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Manual on the application of the HACCP system in mycotoxin prevention and control

FAO FOOD AND NUTRITION PAPER

73

INTERNATIONAL ATOMIC ENERGY AGENCY

Food and Agriculture Organization of the United Nations

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FAO/IAEA Training and Reference Centre for Food and Pesticide Control

INTERNATIONAL
ATOMIC ENERGY
AGENCY

Food
and
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Nations

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FOREWORD

Mycotoxins are considered to be among the most significant food contaminants with regard to their negative impact on public health, food security and the national economy of many countries, particularly the developing ones. They affect a wide range of agricultural products, including cereals, dried fruits, nuts, coffee beans and oilseeds, which are the backbone of most developing economies. These major crops are highly susceptible to fungal contamination and mycotoxin production. Mycotoxin contamination of susceptible commodities occurs as a result of environmental conditions in the field as well as improper harvesting, storage and processing operations.

The Hazard Analysis Critical Control Point (HACCP) system has been increasingly and successfully applied by the food industry and by official food control authorities to prevent and control risks associated with potential contamination of food products with pathogenic micro-organisms and chemical toxicants. Food safety programmes routinely use information about the factors leading to contamination to establish preventive and control procedures, thus providing the consumer with a safe, wholesome food supply.

This Manual was prepared by the Food and Agriculture Organization of the United Nations (FAO) and the International Agency for Atomic Energy (IAEA) through their joint FAO/IAEA Training and Reference Centre for Food and Pesticide Control. Its main aim is to provide guidance to those countries/institutions wishing to apply the HACCP approach to mycotoxin prevention and control.

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The examples presented in this Manual have been based on real case studies and aim to illustrate the application of HACCP specifically for mycotoxin control. It must be emphasised that these HACCP plans are only examples and are for guidance and training only. Every HACCP plan must be developed following the 12 tasks, and applying the seven principles of HACCP as defined by the Codex Alimentarius Commission. It is unlikely that any two HACCP plans will be identical, even for the same product.

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Chapter 1

AN INTRODUCTION TO MYCOTOXINS

WHAT ARE MYCOTOXINS?

"...Wailing and writhing men collapsed in the street: others fell over and foamed in epileptic fits whilst some vomited and showed signs of insanity. Many of them shouted "Fire! I'm burning". It was an invisible fire that separated the flesh from the bones and consumed it. Men, women and children died in unbearable agonising pain."..

These are the words used by a tenth century chronicler to describe a disease which affected many parts of Europe in 943 AD. The disease became known as 'St Anthony's fire' because of the burning sensation experienced by the victims, many of whom visited the shrine of St Anthony in France in the hope of being cured. We now know that St Anthony's Fire (ergotism) was caused by the consumption of rye contaminated with the 'ergot alkaloids', produced by the mould *Claviceps purpurea* (Bove, 1970; Beardall and Miller, 1994), and that it reached epidemic proportions in many parts of Europe in the tenth century. Toxic secondary metabolites, such as the ergot alkaloids, which are produced by certain moulds are described as 'mycotoxins', and the diseases they cause are called 'mycotoxicoses'.

As recently defined by Pitt (1996), mycotoxins are 'fungal metabolites which when ingested, inhaled or absorbed through the skin cause lowered performance, sickness or death in man or animals, including birds.'

It is likely that mycotoxins have plagued mankind since the beginning of organised crop production. It has been surmised, for example, that the severe depopulation of western Europe in the thirteenth century was caused by the replacement of rye with wheat, an important source of *Fusarium* mycotoxins (Miller, 1991). The development of the *Fusarium* toxins in overwintered grain was also responsible for the death of thousands, and the decimation of entire villages, in Siberia during the Second World War. The mycotoxicosis latterly known as 'alimentary toxic aleukia' (ATA) produced vomiting, acute inflammation of the alimentary tract, anaemia, circulatory failure and convulsions.

Mycotoxins occur in a wide variety of foods and feeds and have been implicated (Mayer, 1953; Coker, 1997) in a range of human and animal diseases. Exposure to mycotoxins can produce both acute and chronic toxicities ranging from death to deleterious effects upon the central nervous, cardiovascular and pulmonary systems, and upon the alimentary tract. Mycotoxins may also be carcinogenic, mutagenic, teratogenic and immunosuppressive. The ability of some mycotoxins to compromise the immune response and, consequently, to reduce resistance to infectious disease is now widely considered to be the most important effect of mycotoxins, particularly in developing countries.

The mycotoxins attract world-wide attention because of the significant economic losses associated with their impact on human health, animal productivity and both domestic and international trade. It has been estimated (Miller, Personal communication), for example, that annual losses in the USA and Canada, arising from the impact of mycotoxins on the feed and livestock industries, are of the order of $5 billion. In developing countries, where the food staples (e.g. maize and groundnuts) are susceptible to contamination, it is likely that significant additional losses will occur amongst the human population because of morbidity and premature death associated with the consumption of mycotoxins.

MYCOTOXICOLOGY - A SYSTEMS APPROACH

A 'system' may be viewed as a set of interacting components, where the interactions are just as important as the components themselves (after Open University, 1987). A 'systems' approach to the control of mycotoxins utilises (Coker, 1997) conceptual models of interactions between, and within, commodity, spoilage, mycotoxin, and control subsystems. Within a system, the sub-systems can freely interact; in other words, activity within one subsystem can influence events in one or more other sub-systems.

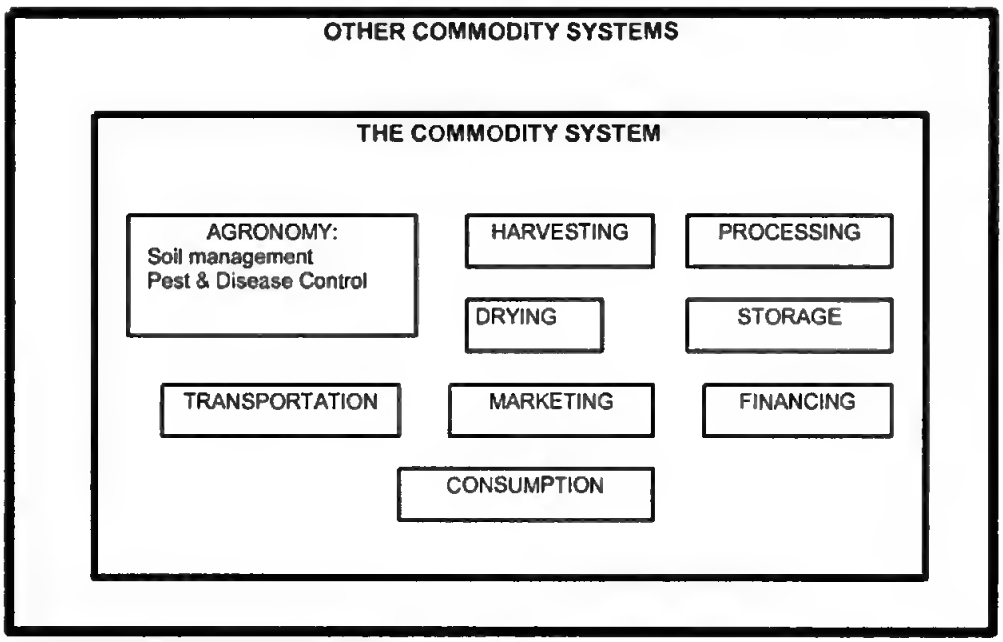
A better understanding of both the interactions and the components associated with these systems will assist in understanding the aetiology of mycotoxin production, and in formulating appropriate interventions for the control of mycotoxins and mycotoxicoses.

THE COMMODITY SYSTEM

Any commodity system is composed of numerous interacting technical and socio-economic 'processes' including, for example, pest and disease control, harvesting, drying, processing, marketing, credit and pricing policies and cultural issues, to name but a few. A generalised,

simplified commodity system is represented in Figure 1 where selected processes are represented as interacting subsystems.

Figure 1. The Commodity System

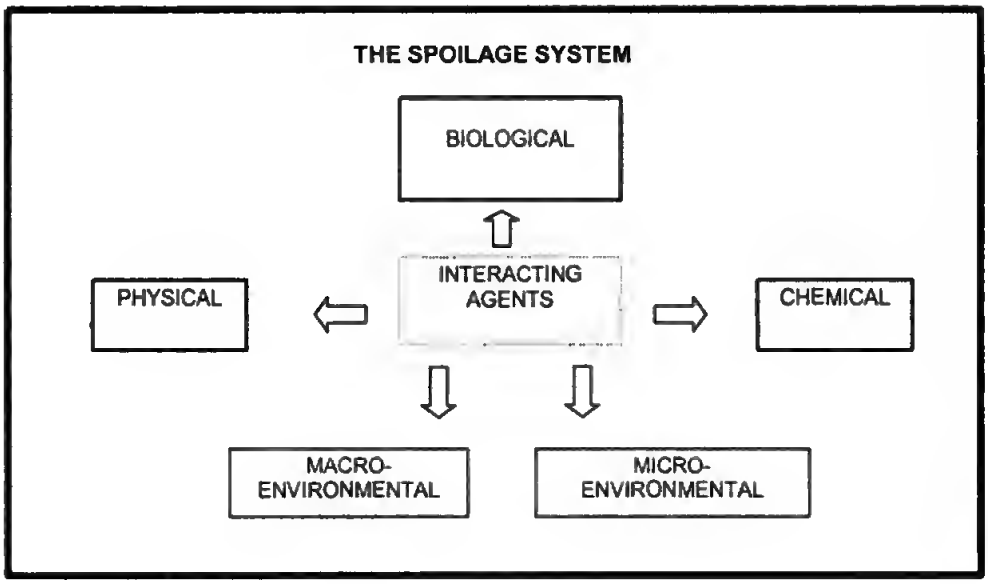


At any point within the commodity system, the condition of the commodity is determined by a complex milieu involving a multitude of interactions between the crop, the macro- and micro-environment and a variety of biological, chemical, physical and socio-economic factors. A change within any one process will invariably bring about changes in one or more of the other processes. Action taken before harvest to control pest damage and/or increase production (e.g selection of varieties, timing of harvest) can have a significant impact on the post-harvest quality of the commodity. Hybrid white maize, for example, has much higher yields than traditional varieties but has poor on-farm storage characteristics. Similarly, since it is very rare for a single commodity system to exist in isolation within a given agro-climatic region, it should be remembered that activities within one system can significantly effect events in other systems. Given the finite resources of farmers, for example, an increase in the importance of one commodity will frequently lead to the allocation of less resources towards the care of other commodities.

THE SPOILAGE SYSTEM

Biodeterioration is the net result of numerous interacting spoilage agents which may be broadly described as biological, chemical, physical, macro-environmental and micro-environmental (Figure 2). However, the relative impact of these agents will often be largely determined by the nature and extent of human intervention.

Figure 2 The Spoilage System



The factors which primarily contribute to bioterioration (including mould growth) within an ecosystem, are moisture, temperature and pests. Moulds can grow over a wide range of temperatures and, in general, the rate of mould growth will decrease with decreasing temperature and available water. In grains, moulds utilise intergranular water vapour, the concentration of which is determined by the state of the equilibrium between free water within the grain (the grain moisture content) and water in the vapour phase immediately surrounding the granular particle. The intergranular water concentration is described either in terms of the equilibrium relative humidity (ERH, %) or water activity (a_w). The latter describes the ratio of the vapour pressure of water in the grain to that of pure water at the same temperature and pressure, whilst the ERH is equivalent to the water activity expressed as a percentage. For a

given moisture content, different grains afford a variety of water activities and, consequently, support differing rates and type of mould growth. Typical water activities which are necessary for mould growth range from 0.70 to 0.99, the water activity, and the propensity for mould growth increasing with temperature. Maize, for example, can be relatively safely stored for one year at a moisture level of 15 per cent and a temperature of 15°C. However, the same maize stored at 30°C will be substantially damaged by moulds within three months.

Insects and mites (arthropods) can also make a significant contribution towards the biodeterioration of grain because of the physical damage and nutrient losses caused by their activity, and also because of their complex interaction with moulds and mycotoxins. The metabolic activity of insects and mites causes an increase in both the moisture content and temperature of the infested grain. Arthropods also act as carriers of mould spores and their faecal material can be utilised as a food source by moulds. Furthermore, moulds can provide food for insects and mites but, in some case, may also act as pathogens.

Another important factor that can affect mould growth is the proportion of broken kernels in a consignment of grain. Broken kernels, caused by general handling and/or insect damage, are predisposed to mould invasion of the exposed endosperm.

Mould growth is also regulated by the proportions of oxygen, nitrogen and carbon dioxide in the inter-granular atmosphere. Many moulds will grow at very low oxygen concentrations; a halving of linear growth, for example, will only be achieved if the oxygen content is reduced to less than 0.14 per cent. Interactions between the gases and the prevailing water activity also influence mould growth.

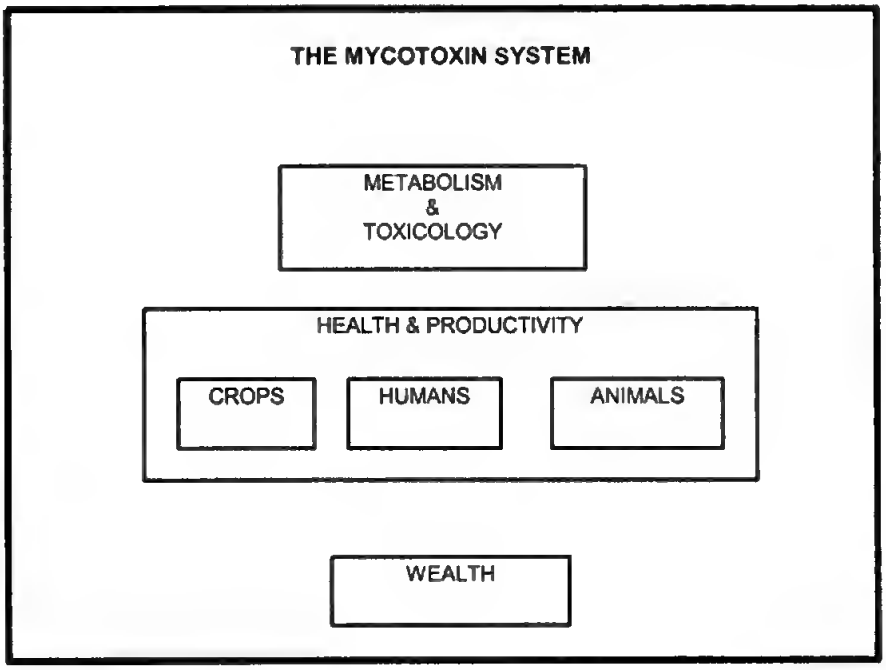
The interactions described above, within granular ecosystems, will support the growth of a succession of micro-organisms, including toxigenic moulds, as the nutrient availability and microenvironment changes with time. In the field, grains are predominantly contaminated by those moulds requiring high water activities (at least 0.88) for growth, whereas stored grains will support moulds which grow at lower moisture levels.

It is well recognised that the main factors which influence the production of mycotoxins are water activity and temperature. However, given the complexity of the ecosystems supporting the production of mycotoxins, the conditions under which toxigenic moulds produce mycotoxins are still poorly defined; and have recently been comprehensively reviewed (ICMSF, 1996).

THE MYCOTOXIN SYSTEM

The Mycotoxin System (Figure 3) may be considered in terms of three interacting subsystems: metabolism & toxicology; health & productivity; and wealth. After exposure (by ingestion, inhalation or skin contact), the toxicity of a mycotoxin is determined by a sequence of events (metabolism) involving the administration, absorption, transformation, pharmacokinetics, molecular interactions, distribution, and excretion of the toxin and its metabolites. In turn, the toxicity of a mycotoxin will be manifested by its effect on the health and productivity of crops, humans and animals; and, these effects will influence the production of wealth associated with human endeavour and agricultural and livestock products.

Figure 3 The Mycotoxin System



Mycotoxins of world-wide Importance

Those moulds and mycotoxins which are currently considered to be of world-wide importance (Miller, 1994) are shown in Table 1 and Figure 4.

An 'important' mycotoxin will have demonstrated its capacity to have a significant impact upon human health and animal productivity in a variety of countries.

Table 1 - Moulds and mycotoxins of world-wide importance

Mould species	Mycotoxins produced
Aspergillus parasiticus	Aflatoxins B_1, B_2, G_1, G_2
Aspergillus flavus	Aflatoxins B_1, B_2
Fusarium sporotrichioides	T-2 toxin
Fusarium graminearum	Deoxynivalenol (or nivalenol)
	Zearalenone
Fusarium moniliforme (F. verticillioides)	Fumonisin B_1
Penicillium verrucosum	Ochratoxin A
Aspergillus ochraceus	Ochratoxin A

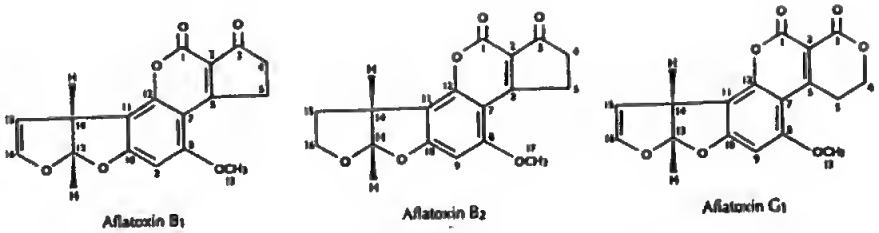
The Aflatoxins

The optimal water activity for growth of *A. flavus* is high (about 0.99). The maximum is at least 0.998 whereas the minimum water activity for growth has not been defined precisely. Pitt and Miscamble (1995) report a minimum of approximately 0.82. In general, production of toxins appears to be favoured by high water activity. *A. flavus* is reported to grow within the temperature range 10 - 43°C. The optimal growth rate occurs at a little above 30°C, reaching as much as 25 mm per day. The aflatoxins are produced by *A. flavus* over the temperature range 15 - 37°C, at least. It is not possible to specify an optimum temperature for the production of the toxins, although production between 20 - 30°C is reported to be significantly greater than at higher and lower temperatures.

The effect of water activity and temperature on the behaviour of *A. parasiticus* is similar to that described above for *A. flavus*. Pitt and Miscamble (1995) have reported a minimum for growth of about 0.83; and a minimum for aflatoxin production of about 0.87. There are only limited data on the effect of temperature on the growth of *A. parasiticus* and the production of the aflatoxins. It was reported that optimal growth and toxin production occur at approximately 30 and 28°C, respectively.

The term 'aflatoxins' was coined in the early 1960s when the death of thousands of turkeys ('Turkey X' disease), ducklings and other domestic animals was attributed to the presence of *A. flavus* toxins in groundnut meal imported from South America (Austwick, 1978).

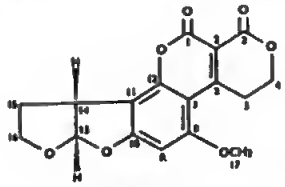
Figure 4 - Some Important Mycotoxins



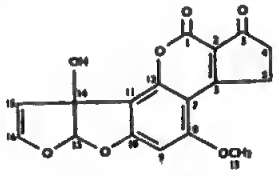
Aflatoxin B_1

Aflatoxin B_2

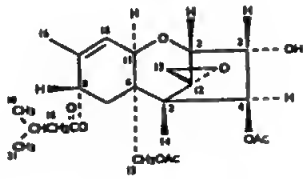
Aflatoxin G_1



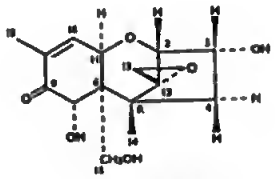
Aflatoxin G_2



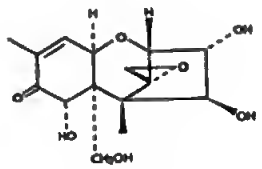
Aflatoxin M_1



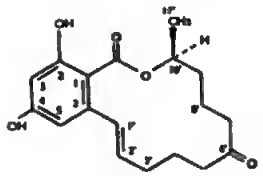
T-2 toxin



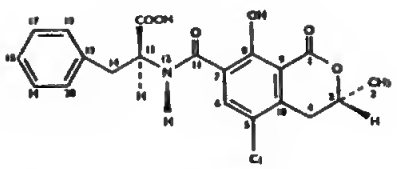
Deoxynivalenol



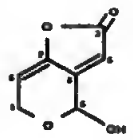
Nivalenol



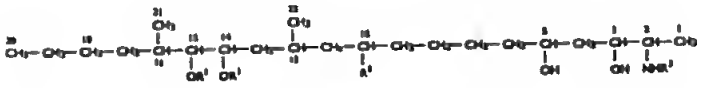
Zearalenone



Ochratoxin A



Patulin



Fumonisins

A_1: R^1 = $COCH_2CH(CO_2H)CH_2CO_2H$; R^2 = OH; R^3 = $C^{19}OC^{34}H_{69}$
A_2: R^1 = $COCH_2CH(CO_2H)CH_2CO_2H$; R^2 = H; R^3 = $COCH_3$
B_1: R^1 = $COCH_2CH(CO_2H)CH_2CO_2H$; R^2 = OH; R^3 = H
B_2: R^1 = $COCH_2CH(CO_2H)CH_2CO_2H$; R^2 = R^3 = H

(Although the aflatoxins are the major toxins associated with this mycotoxicosis, another mycotoxin - cyclopiazonic acid (Figure 5) - has been implicated (Bradburn *et al.*, 1994) in the aetiology of Turkey X disease.) The chronic effects of low dietary levels (parts per billion) of aflatoxin on livestock are also well documented (Coker, 1997) and include decreased productivity and increased susceptibility to disease.

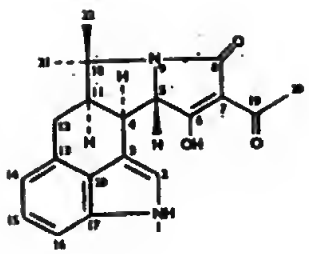
The aflatoxin-producing moulds occur widely, in temperate, sub-tropical and tropical climates, throughout the world; and the aflatoxins may be produced, both before and after harvest, on many foods and feeds especially oilseeds, edible nuts and cereals (Coker, 97).

Although the aflatoxins are predominantly associated with commodities of sub-tropical and tropical origin, their occurrence has also been reported (Pettersson *et al*, 1989) in temperate climates in acid-treated grains.

Aflatoxin B_1 is a human carcinogen (IARC, 1993a) and is one of the most potent hepatocarcinogens known. Human fatalities have also occurred (Krishnamachari *et al.*, 1975) from acute aflatoxin poisoning in India (in 1974), for example, when unseasonal rains and a scarcity of food prompted the consumption of heavily contaminated maize. If the immunosuppressive action of the aflatoxins in livestock is similarly manifested in humans, it is possible that the aflatoxins (and other mycotoxins) could play a significant role in the aetiology of human disease in some developing countries, where a high exposure to these toxins has been reported.

Lubulwa and Davis (1994) have studied economic losses attributable to the occurrence of aflatoxin only, in maize and groundnuts, in Southeast Asian countries (Thailand, Indonesia and the Philippines). They concluded that contaminated maize accounted for about 66 per cent of the total loss, whereas losses attributable to spoilage and deleterious effects on human and animal health were 24, 60 and 16 per cent of the total, respectively. However, the study considered losses associated with morbidity and premature death caused by cancer only. Consequently, it is likely that when the additional effects on human health caused by the immunotoxic effect of aflatoxin (and other mycotoxins) are
included, the loss associated with aflatoxins will be significantly increased.

Figure 5 The Structure of Cyclopiazonic Acid



The Trichothecenes

Surprisingly little is known about the effects of water activity and temperature on the behaviour of the *Fusarium* moulds, including the production of mycotoxins.

In the case of *F. graminearum*, the temperature limits for growth have not been reported, although the optimal temperature has been estimated at 24 - 26 °C. The minimum water activity for growth is 0.9; the maximum limit is recorded as in excess of 0.99. No information is available on the effect of water activity and temperature on the production of deoxynivalenol, nivalenol and zearalenone.

The minimum water activity for the growth of *F. sporotrichioides* is 0.88, whereas the maximum limit is reported as >0.99. The minimum, optimal and maximum temperatures for growth are - 2.0, 22.5 - 27.5 and 35°C, respectively. As with the other *Fusarium* moulds, there is no information on the conditions required for the production of T-2 toxin.

T-2 toxin and deoxynivalenol (Figure 4) belong to a large group of structurally-related sesquiterpenes known as the 'trichothecenes'.

T-2 toxin is produced on cereals in many parts of the world and is particularly associated with prolonged wet weather at harvest. It is the probable cause of 'alimentary toxic aleukia' (ATA), a disease (IARC, 1993b) which affected thousands of people in Siberia during the Second World War, leading to the elimination of entire villages. The symptoms of ATA included fever, vomiting, acute inflammation of the alimentary tract and a variety of blood abnormalities. T-2 toxin is responsible for outbreaks of haemorrhagic disease in animals and is associated with the formation of oral lesions and neurotoxic effects in poultry. The most significant effect of T-2 toxin (and other trichothecenes) is the immunosuppressive activity which has been clearly demonstrated in experimental animals; and which is probably linked to the inhibitory effect of this toxin on the biosynthesis of macromolecules. There is limited evidence that T-2 toxin may be carcinogenic in experimental animals.

Deoxynivalenol (DON) is probably the most widely occurring *Fusarium* mycotoxin, contaminating a variety of cereals, especially maize and wheat, in both the developed and developing world. The outbreaks of emetic (and feed refusal) syndromes amongst livestock,

caused by the presence of DON in feeds, has resulted in the trivial name, vomitoxin, being attributed to this mycotoxin.

The ingestion of DON has caused outbreaks (IARC, 1993c; Bhat *et al.*, 1989; Luo, 1988) of acute human mycotoxicoses in India, China and rural Japan. The Chinese outbreak, in 1984-85, was caused by mouldy maize and wheat; symptoms occurred within five to thirty minutes and included nausea, vomiting, abdominal pain, diarrhoea, dizziness and headache.

To date, nivalenol-producing isolates of *F. graminearum* have been observed, on rice and other cereals, only in Japan and have been associated with the occurrence of red mould disease ('Akakabi-byo'). Symptoms include anorexia, nausea, vomiting, headache, abdominal pain, diarrhoea and convulsions (Marasas *et al.*, 1984).

Zearalenone

Zearalenone is a widely distributed oestrogenic mycotoxin occurring mainly in maize, in low concentrations, in North America, Japan and Europe. However, high concentrations can occur in developing countries, especially when maize is grown under more temperate conditions in, for example, highland regions.

Zearalenone is co-produced with deoxynivalenol by *F. graminearum* and has been implicated, with DON, in outbreaks of acute human mycotoxicoses.

Exposure to zearalenone-contaminated maize has caused (Udagawa, 1988) hyperoestrogenism in livestock, especially pigs, characterised by vulvar and mammary swelling and infertility. There is limited evidence in experimental animals for the carcinogenicity of zearalenone.

The Fumonisins

The fumonisins are a group of recently characterised mycotoxins produced by *F. moniliforme*, a mould which occurs worldwide and is frequently found in maize (IARC, 1993d). Fumonisin B_1 has been reported in maize (and maize products) from a variety of agroclimatic regions including the USA, Canada, Uruguay, Brazil, South Africa, Austria, Italy and France. The toxins especially occur when maize is grown under warm, dry conditions.

The minimum water activity for the growth of *F. moniliforme* is 0.87; the maximum limit is recorded as >0.99. The minimum, optimal and maximum temperatures for growth are 2.5 - 5.0, 22.5 - 27.5 and 32 –37°C, respectively. There is no information on the conditions required for the production of fumonisin B_1.

Exposure to fumonisin B_1 (FB1) in maize causes leukoencephalomalacia (LEM) in horses and pulmonary oedema in pigs. LEM has been reported in many countries including the USA, Argentina, Brazil, Egypt, South Africa and China. FB1 is also toxic to the central nervous system, liver, pancreas, kidney and lung in a number of animal species.

The presence of the fumonisins in maize has been linked with the occurrence of human oesophageal cancer in the Transkei, southern Africa and China. The relationship between exposure to *F. moniliforme*, in home-grown maize, and the incidence of oesophageal cancer has been studied in the Transkei during the ten-year period 1976-86 (Rheeder et al, 1992). The percentage of kernels infected by *F. moniliforme* was significantly higher in the high-risk cancer area during the entire period; and FB1 and FB2 occurred at significantly higher levels in mouldy maize obtained from high-risk areas in 1986.

Previously, an evaluation by the International Agency for Research on Cancer had concluded that there is sufficient evidence in experimental animals for the carcinogenicity of cultures of *F. moniliforme* that contain significant amounts of the fumonisins; whereas there is limited evidence, in experimental animals, for the carcinogenicity of fumonisin B_1 (IARC, 1993d). However, the results of a recently completed study of the toxicology and carcinogenesis of fumonisin B_1 has been reported (NTP, 1999) by the National Toxicology Program of the US Department of Health and Human Services. Although the report is still in draft form, it concludes that there is clear evidence of carcinogenic activity of fumonisin B_1 in male F344/N rats based on the increased incidences of renal tubule neoplasms; and that there is also clear evidence of carcinogenic activity of fumonisin B_1 in female $B6C3F_1$ mice based on the increased incidences of hepatocellular neoplasms. There is no evidence of carcinogenic activity of fumonisin B_1 in female rats or male mice.

Ochratoxin A

A. ochraceus grows more slowly than both *A. flavus* and *A. parasiticus*, but can grow at a water activity as low as 0.79. Growth has also been reported within the temperature range 8 - 37 °C,

with an optimum variously reported as 25 - 31°C. Ochratoxin A is produced within the temperature range 15 - 37°C, with an optimal production at 25 - 28°C.

P. verrucosum grows within the temperature range 0 - 31°C and at a minimum water activity of 0.80. Ochratoxin A is produced over the whole temperature range. Significant quantities of toxin can be produced at a temperature as low as 4°C, and at a water activity as low as 0.86.

Exposure (IARC, 1993e) to ochratoxin A (OA) appears to occur mainly in wheat and barley growing areas in temperate zones of the northern hemisphere. The levels of OA reported in these commodities ranges from trace amounts to 6000 μg/kg, in Canadian wheat. In the UK, reported levels have varied from <25 to 5,000 and from <25 to 2,700 μg/kg in barley and wheat respectively. It also occurs in maize, rice, peas, beans, cowpeas, vine fruits and their products, coffee, spices, nuts and figs.

The ability of OA to transfer from animal feeds to animal products has been demonstrated by the occurrence of this toxin in retail pork products, and the blood of swine, in Europe.

Although cereal grains are considered to be the main human dietary source of OA , it has been suggested (IARC, 1993e) that pork products may also be a significant source of this toxin. Ochratoxin A has been found in blood (and milk) from individuals in a variety of European countries, including France, Italy, Germany, Denmark, Sweden, Poland, Yugoslavia and Bulgaria. One of the highest reported levels is 100 ng/ml OA in blood from Yugoslavia (Fuchs et al, 1991); whereas 6.6 ng/ml OA in milk has been recorded in Italy (Micco et al, 1991).

Existing or proposed regulations for OA are available in at least eleven countries, the permitted levels ranging from 1 to 50 μg/kg in foods and from 100 to 1000 μg/kg in feeds. In Denmark, the acceptability of pork products from a specific carcass is determined by analysing the OA content of the kidney. The pork meat and certain organs can be consumed as food if the OA content of the kidney is no more than 25 and 10 μg/kg respectively (van Egmond, 1997).

A provisional tolerable weekly intake of 100 ng/kg bw, of OA, approximating to 14 ng/kg body weight per day, has been recommended by a WHO/FAO Joint Expert Committee on Food Additives, JECFA (JECFA, 1996a).

Ochratoxin A has been linked with the human disease Balkan endemic nephropathy, a fatal, chronic renal disease occurring in limited areas of Bulgaria, the former Yugoslavia and Romania.

OA causes renal toxicity, nephropathy and immunosuppression in several animal species and it is carcinogenic in experimental animals.

There is sufficient evidence in experimental animals for the carcinogenicity of OA (IARC, 1993e).

Patulin

Patulin (Figure 4) is an antibiotic produced by a number of moulds. It occurs in rotten apples contaminated by *Penicillium expansum* and, consequently, may occur in apple juice and other apple-based products.

Experimental studies have demonstrated that patulin is a neurotoxin and that it produces marked pathological changes in the viscera. Although patulin has been reported as inducing local sarcomas, no mutagenic activity has been discernible in most short-term tests.

JECFA (JECFA, 1996b) has established a provisional maximum tolerable daily intake of 400 ng/kg bw for patulin.

The co-occurrence of mycotoxins

The complex ecology of mould growth and mycotoxin production can produce mixtures of mycotoxins in foods and feeds, especially in cereals. The co-occurrence of mycotoxins can affect (Miller, 1991) both the level of mycotoxin production and the toxicity of the contaminated material. The production of the aflatoxins in stored grains, for example, may be enhanced by the presence of trichothecenes, whereas the toxicology of naturally occurring combinations of trichothecene mycotoxins is reportedly (Schiefer et al, 1986) determined by synergistic interactions, in experimental animals. For example, in a study with swine, the affect of deoxynivalenol on weight gain and feed conversion was synergized by T-2 toxin. Interactions involving non-toxic fungal metabolites have also been reported (Dowd, 1989), including the potent synergism of non-toxic *F. graminearum* metabolites (culmorin, dihydroxycalonectrin and sambucinol) with deoxynivalenol. To date, too little is known about this particularly important area of mycotoxicology.

Mycotoxins of Regional Importance

There are a number of mycotoxicoses which are not widely occurring, but which are of importance to the exposed populations in the affected regions. Mycotoxicoses which fall into this category (Table 2) include those associated with moulds occurring in both growing and stored forage crops. The moulds and mycotoxins include those which have been associated with a variety of livestock diseases including ergotism, paspalum staggers, ryegrass staggers, facial eczema, fescue foot, lupinosis, slobber syndrome and stachybotryotoxicosis (Lacey, 1991).

Table 2. Moulds and mycotoxins of regional importance

Mould species	Mycotoxins produced	Mycotoxicosis
Claviceps purpurea	Ergotamine alkaloids	Ergotism
Claviceps fusiformis	Clavine alkaloids	Ergotism
Claviceps paspali	Paspalinine	*Paspalum* staggers
Acremonium loliae	Lolitrem	Ryegrass staggers
Balansia spp?	Alkaloids?	Fescuefoot
Pithomyces chartarum	Sporidesmin	Facial eczema
Phomopsis leptostromiformis	Phomopsin	Lupinosis
Rhizoctonia leguminicola	Slaframine	Slobber syndrome
Stachybotrys atra	Satratoxins	Stachybotryotoxicosis
Diplodia maydis	Diplodiatoxin	Diplodiosis

Most farm animals consume pasture crops, either by grazing on the living pasture or by consuming the crops as hay or silage. The crops can be colonized by moulds throughout this period, the development of the moulds and the production of fungi being dependent on the prevailing ecosystem. Growing crops present a variety of micro-environments. For example, the uppermost leaves of a plant will be subjected to extreme fluctuations of temperature and relative humidity, whereas those leaves towards the base of the plant will present a more shaded, moderate, humid environment; the surface texture of the leaf will also effect the micro-environment.

THE SOCIO-ECONOMIC SYSTEM

The socio-economic system describes those social (eg cultural, political) and economic (macro- and micro) factors which will exert an important influence on events within the mycotoxicology system; and which should be most thoroughly addressed when any attempt is made to control the production of moulds and mycotoxins. In some instances, given the complexity and unpredictability of human behaviour, it can be very difficult to intervene successfully within the socio-economic system. However, technical interventions which are designed to alleviate spoilage will only be successfully implemented if they can be accommodated and exploited within the existing socio-economic system. Whenever efforts are made to improve the quality of foods and feeds, it should be clearly established that there is a definite need for a better quality product, and that the community is prepared to bear any associated increase in the cost of the improved commodity.

THE CONTROL SYSTEM

The successful management of interacting commodity systems *('commodity management')* requires the co-ordinated inputs of an *inter*disciplinary team', where the potential advantages arising from the dynamics of the team are realised by fully exploiting the *interactions* between the skills, disciplines and backgrounds of the individual team members. The team will have the skills required to enable it to operate across commodity systems, identifying those factors which are compromising the quality of the products, and introducing appropriate interventions.

The Control System (Figure 6) illustrates a selection of preventative and curative interventions (measures) which may be utilised for the control of mycotoxins, once the nature of the contamination process has been properly evaluated.

Those factors which are compromising the quality of the products of the commodity system, and leading to the production of moulds and mycotoxins, may be evaluated by the implementation of: carefully designed surveillance studies; recently developed biomonitoring methods, to measure the exposure to mycotoxins of individuals; and socio-economic studies, which address a variety of social, marketing and financial issues (Coker, 1997). The occurrence of moulds and mycotoxins can be alleviated by the application of a variety of preventative measures both before and after harvest including, for example, appropriate pest and disease control measures and good harvesting, drying and storage practices. Once mycotoxin contamination has occurred, it can be

alleviated by a variety of predominantly post-harvest measures including processing, detoxification, and segregation (Coker, 1997; FAO, 1999).

A structured, systematic approach to the control of mycotoxins is required, focusing upon the need for preventative control measures, and recognising the intimate interactions that occur throughout commodity systems and related systems.

INTEGRATION OF SYSTEMS

Hazard Analysis and Critical Control Point (HACCP) is a food safety management system that is based upon the systematic identification and assessment of hazards in foods, and the definition of means to control them. It is an important component of an integrated approach to food safety. The inter-relationship of HACCP with other food safety tools is illustrated in Figure 7.

Chapters 2 and 3 describe the adoption of HACCP as a means of effecting the systematic control of mycotoxins, culminating in case studies addressing the control of particular mycotoxin problems.

Figure 6 - The Control System

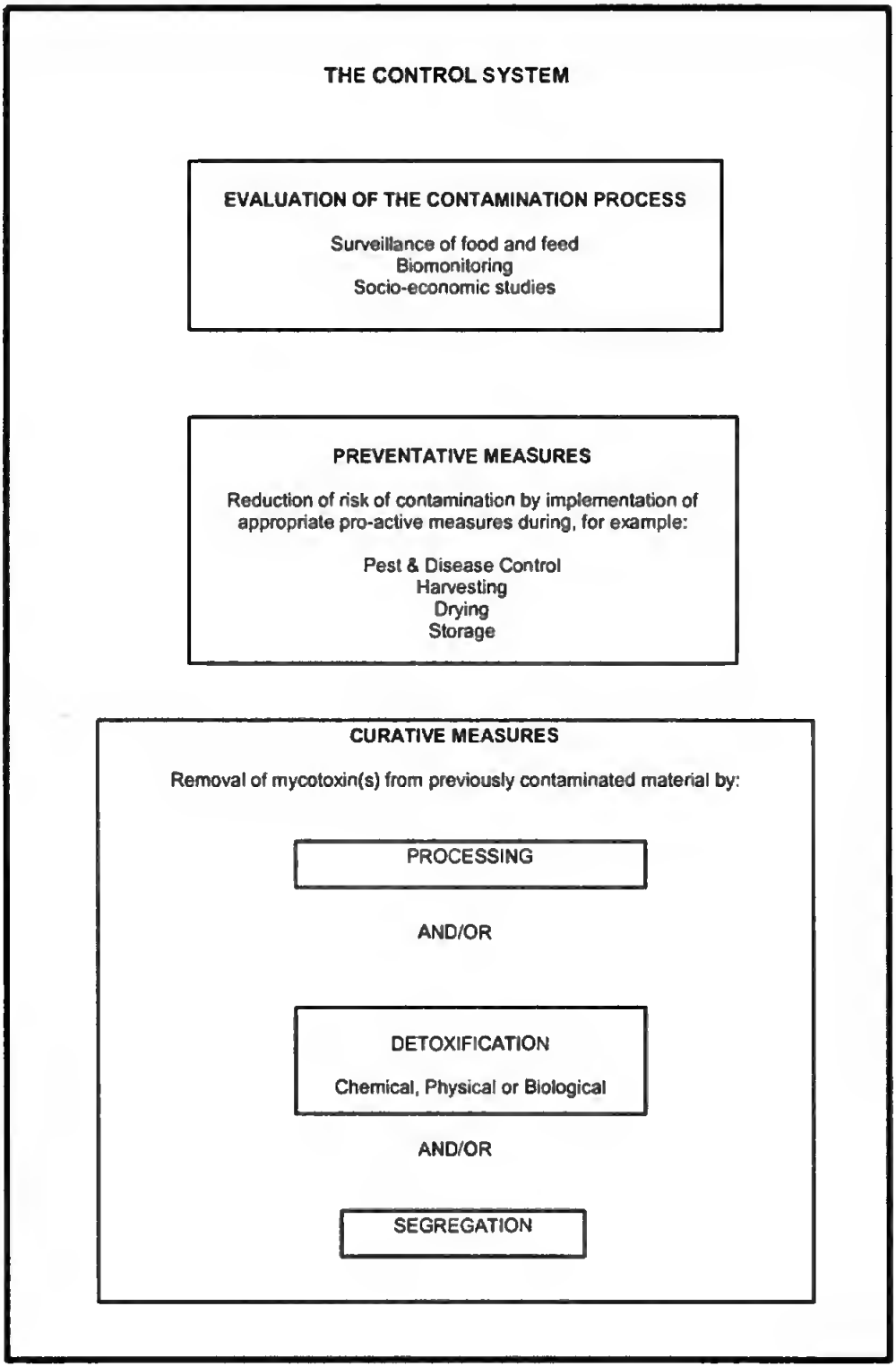
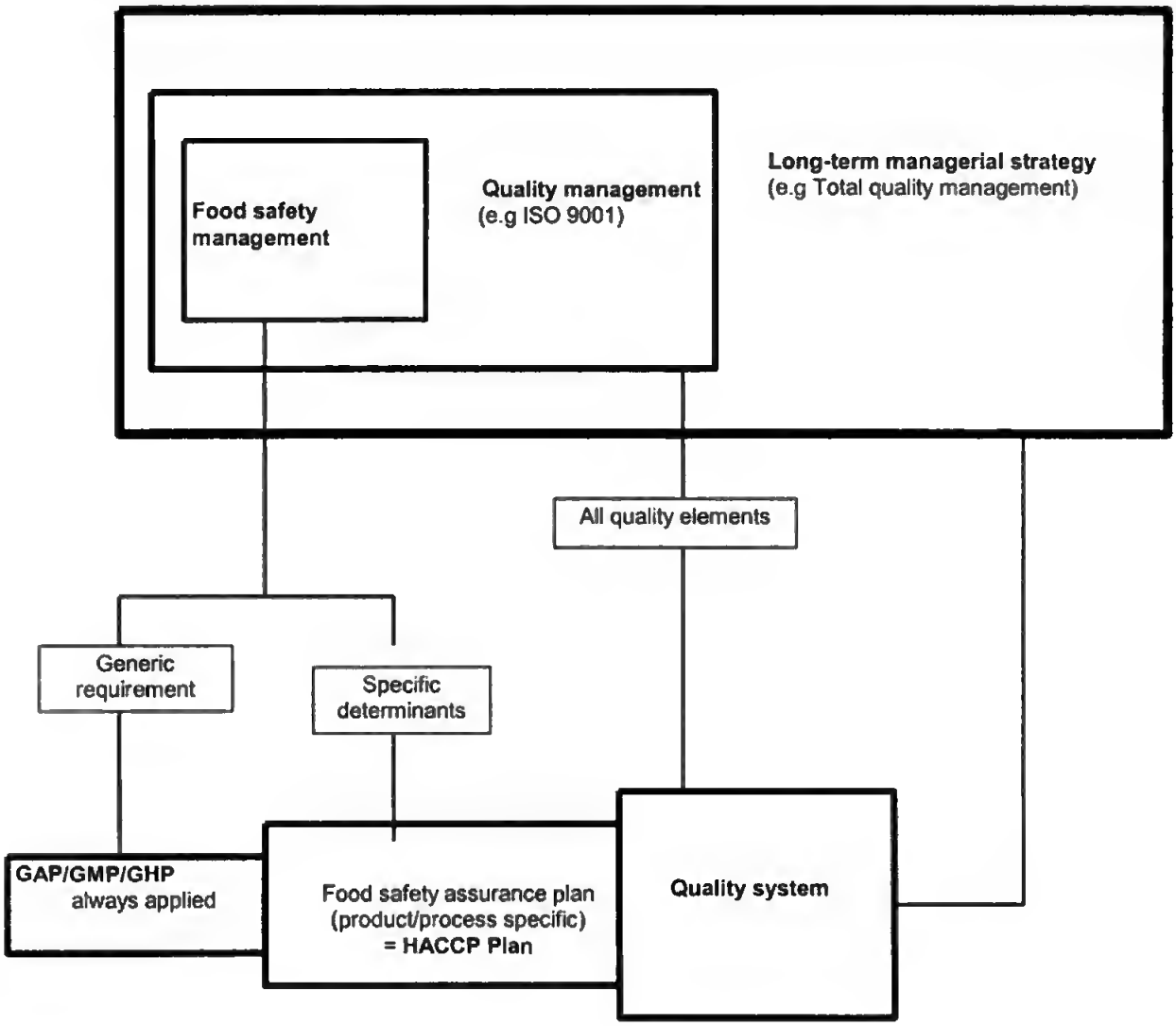


Figure 7 - Food safety tools: an integrated approach



After Food Safety Management Tools (Jouve 1998)

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Chapter 2

AN OVERVIEW OF HAZARD ANALYSIS AND CRITICAL CONTROL POINT (HACCP)

"An ounce of prevention is worth a pound of cure"

INTRODUCTION

HACCP was originally developed as a microbiological safety system in the early days of the US manned space programme in order to guarantee the safety of astronauts' food. Up until that time most food safety systems were based on end product testing and could not fully assure safe products as 100% testing was impossible. A pro-active, process-focused system was needed and the HACCP concept was born.

The original system was designed by the Pilsbury Company working alongside NASA and the US army laboratories at Natick. It was based on the engineering system Failure, Mode and Effect Analysis (FMEA) which looked at what could potentially go wrong at each stage in the operation along with possible causes and the likely effect, before applying effective control mechanisms.

HACCP is a system that identifies, evaluates and controls hazards which are significant for food safety. It is a structured, systematic approach for the control of food safety throughout the commodity system, from the plough to the plate. It requires a good understanding of the relationship between cause and effect in order to be more pro-active and it is a key element in Total Quality Management (TQM). HACCP builds on the foundations of well established quality management systems such as Good Manufacturing Practice (GMP), Good Hygienic Practice (GHP), Good Agricultural Practice (GAP), and Good Storage Practice (GSP). The HACCP concept has been successfully applied in the control of quality as well as safety in low-acid canned foods in the USA, and many food companies in Europe and the USA have adopted the approach. Increasingly, regulatory bodies have recognised the usefulness of this tool and its 'principles' have has been incorporated into legislative requirements by both the EU (in the General Hygiene regulations for managing food safety (93/43/EEC)), and the United States Federal Department of Agriculture (CPR - 123). The National Advisory Committee on

Microbiological Criteria for Foods (NACMCF) provided guidelines on HACCP including generic plans and decision trees in 1992, and the Codex Alimentarius Commission adopted the HACCP system at its twentieth session in 1993. HACCP systems can be incorporated into other quality assurance systems such as the ISO 9000 series (Figure 7).

Although conceived as a food safety system for both the agricultural and processing systems, it is in the latter that HACCP has found most application hitherto. This is primarily because it is much easier to apply a HACCP system in a factory where there is a single management or 'owner', and where it is possible to completely prevent a food safety hazard, or eliminate, or reduce it to an acceptable level. In the commodity system there are often many disparate 'owners' of the commodity as it passes from the farm to the consumer, and complete control may be unobtainable. This Manual aims to address this subject, basing the approach as closely as possible on the Codex Code of General Principles on Food Hygiene (1997), which emphasises the importance of GMP/GAP/GHP as sound foundations to incorporate the HACCP approach and develop a user friendly Food Safety Management System.

PRE-REQUISITE PROGRAMMES

Pre-requisite programmes such as GAP, GMP and GHP must be working effectively within a commodity system before HACCP is applied . If these pre-requisite programmes are not functioning effectively then the introduction of HACCP will be complicated, resulting in a cumbersome, over-documented system.

Good Agricultural Practices

Primary Production

Primary food production should be managed to ensure that food is safe and wholesome for the consumer. Production will start on the farm, in the sea or lake or even within a forest. It is essential that certain ground rules are followed. Land used for crop or horticulture production should be fit for purpose and should not have previously been contaminated with heavy metals, industrial chemicals or environmental waste. Such hazards will be transferred into the food chain rendering the commodity unfit for human consumption. Farmers should control production so that contamination of the crop, proliferation of pests, and diseases of animals and plants, do not compromise food safety. Good Agricultural Practices (GAP), including Good Hygienic Practices (GHP) where appropriate, should be adopted to make sure that the harvested commodity will not present a food hazard to the consumer.

Good Storage Practices (GSP) should be followed when the commodity is stored on the farm. As well as being covered in Food Hygiene Basic Texts (CODEX) there are also four ISO procedures that cover the storage of cereal s and pulses (ISO 6322 series). GSP should also be followed for storage throughout the commodity system.

Good Manufacturing Practices

Establishment Design and Facilities

The structure and location of a processing plant needs to be considered in relation to the nature of operations and risks associated with them.

- Food premises should be designed to minimise possibilities of contamination of commodity or product.
- Design and layout should permit maintenance, cleaning and disinfection of the site to minimise airborne contamination.
- All surfaces that come into contact with food should be non toxic, as well as being easy to maintain and clean in order to prevent any additional contamination .
- Suitable facilities should exist for temperature and humidity control, when required.
- Effective measures should exist to prevent access by pests

Control of Operation

Effective control measures should be in place to reduce the risk of contamination of the commodity or food supply such that it is safe and fit for purpose:

- Adequate time, temperature or humidity controls
- Food grade packaging
- Potable water supplies
- Maintenance of equipment

Maintenance and Sanitation

Procedures and work instructions should exist to demonstrate an adequate level of maintenance of an establishment as well as efficient practices for cleaning, waste management, and pest control. Overall, these operations will support the ongoing control of potential food hazards that may contaminate food.

Personnel Hygiene

Measures need to be in place to ensure that food handlers do not contaminate food. This objective can be attained by maintaining an appropriate level of personal cleanliness and following guidelines for personal hygiene.

Transportation

The method of transportation should be such that measures are taken to prevent any contamination or deterioration of the commodity. Commodities or product that need to be transported in certain environments should be appropriately controlled, e.g. chilled, frozen, or stored under specific humidity levels.

Containers and conveyors used for transporting food need to be maintained in good condition and be easy to clean.

Containers used for bulk transfer should be designated and marked specifically for food use only.

Training

All food handlers should be trained in personal hygiene, as well as in the specific operation with which they are working, to a level commensurate with their duties. Food handlers should also be supervised by trained supervisors.

An ongoing training programme for food handlers is paramount to the success of a Food Safety Management System

Product Information and Consumer Awareness

The end product should be accompanied by adequate information to ensure that personnel at the next stage in the food chain will handle, store, process, prepare and display the product safely. Since the consumer may be responsible for performing the ulitimate control measure, the cooking of raw meat or fish, they should have all the relevant information required to carry out this step effectively.

All batches of food should be easily identified, by a batch or lot number, to allow traceability of the commodity if required.

BASIC PRINCIPLES OF HACCP

There are seven discrete activities that are necessary to establish, implement and maintain a HACCP plan, and these are referred to as the 'seven principles' in the Codex Guideline (1997).

The seven principles are[1]:

Principle 1

Conduct a hazard analysis

Identify hazards and assess the risks associated with them at each step in the commodity system. Describe possible control measures.

Principle 2

Determine the Critical Control Points (CCPs)

A critical control point is a step at which control can be applied and is essential to prevent or eliminate a food safety hazard, or reduce it to an acceptable level. The determination of a CCP can be facilitated by the application of a decision tree, such as the one given in Appendix IV.

Principle 3

Establish critical limits.

Each control measure associated with a CCP must have an associated critical limit which separates the acceptable from the unacceptable control parameter.

Principle 4

Establish a monitoring system

Monitoring is the scheduled measurement or observation at a CCP to assess whether the step is under control, i.e. within the critical limit(s) specified in Principle 3.

Principle 5

Establish a procedure for corrective action, when monitoring at a CCP indicates a deviation from an established critical limit.

Principle 6

Establish procedures for verification to confirm the effectiveness of the HACCP plan.

Such procedures include auditing of the HACCP plan to review deviations and product dispositions, and random sampling and checking to validate the whole plan.

Principle 7

Establish documentation concerning all procedures and records appropriate to these principles and their application

DEVELOPING A HACCP PLAN

There are twelve tasks required to develop a HACCP plan and these are designed to ensure that the seven principles are applied correctly. Principle 1, which is to conduct a hazard analysis, requires that the first five tasks have all been addressed in a logical and honest manner so that all real hazards associated with the commodity have been identified. The twelve tasks are discussed briefly below, and listed in Appendix II.

Task 1 – Establish a HACCP team

To fully understand the commodity system and be able to identify all likely hazards and CCPs, it is important that the HACCP team is made up of people from a wide range of disciplines. The team should include:

- A team leader to convene the group and to direct the work of the team ensuring that the concept is properly applied. This person must be familiar with the technique, be a good listener and allow all participants to contribute.

- A specialist with a detailed knowledge of the commodity system is required. This specialist will have a major role in the production of the commodity flow diagrams.

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[1] please refer to Appendix 1 for a definition of the terms used in this section

- Several specialists, each with an understanding of particular hazards and associated risks, e.g. a microbiologist, a chemist, a mycotoxicologist, a toxicologist, a QC manager, a process engineer.

- People, such as packaging specialists, raw material buyers, distribution staff or production staff, farmers, brokers, who are involved with the process, and have working knowledge of it, may be brought into the team temporarily in order to provide relevant expertise.

- The team's progress and results of the analysis should be recorded by a technical secretary.

If any changes are made to composition or operational procedures, it will be necessary to re-assess the HACCP plan in the light of the changes.

The first activity of the HACCP team is to identify the scope of the study. For example, will the whole commodity system be covered, or only selected components? This will make the task more manageable and specialists can be added to the team as and when they are required.

Task 2 - Describe the product

To start a hazard analysis, a full description of the product, including customer specification, should be prepared using a form such as that given in Appendix III. This should include information relevant to safety, e.g. mycotoxin regulation/ target level, composition, physical/chemical properties of the raw materials and the final product, the amount of water available for microbial growth (a_w), the amount of acid or alkali in the product (pH). Also information regarding how the product is to be packaged, stored and transported should also be considered together with facts regarding its' shelf life and recommended storage temperatures. Where appropriate, labelling information and an example of the label should be included. This information will help the HACCP team to identify 'real' hazards associated with the process.

Task 3 - Identify the product's intended use

How the product is intended to be used is an important consideration. Information on whether the product will be consumed directly, or be cooked, or be further processed, will all have a bearing on the hazard analysis , see task 6). The nature of the target group for the product may also be relevant, particularly if it includes susceptible groups such as infants, the elderly, and the

malnourished. The likelihood of misuse of a product should also be considered, such as the use of pet food as a human food, either by accident or design. This information can be recorded on the same form as the product description, see Appendix III.

Task 4 – Draw up the commodity flow diagram

The first function of the team is to draw up a detailed commodity flow diagram (CFD) of the commodity system, or that part of it which is relevant. The expertise of the commodity specialist is important at this stage. Commodity systems will differ in detail in different parts of the world, and even within one country there may be a number of variants. Secondary processing will need to be detailed for each factory, using generic flows only as a guide. Examples of commodity flow diagrams are included in the case studies presented in Chapter 3.

Task 5 - On site confirmation of flow diagram

Upon completion of the CFD, members of the team should visit the commodity system (e.g. farm, store or manufacturing area) to compare the information present on the CFD with what actually happens in practice. This is known as "walking the line", a step by step practice to check that all information regarding materials, practices, controls etc. have been taken into consideration by the team during the preparation of the CFD. Information such as time of harvest, drying procedures, storage conditions, the marketing chain, socio-economic factors, grading systems and any incentive for improved quality or safety, and processing systems, should be collected and included in the CFD as appropriate. The site for which the HACCP plan is being designed should be visited as many times as possible to ensure that all relevant information has been collected.

Task 6 – Identify and analyse hazard(s) - (Principle 1)

Effective hazard identification and hazard analysis are the keys to a successful HACCP Plan. All real or potential hazards that may occur in each ingredient and at each stage of the commodity system should be considered. Food safety hazards for HACCP programmes have been classified into three types of hazards:

- Biological: typically foodborne bacterial pathogens such as *Salmonella*, *Listeria* and *E. coli*, also viruses, algae, parasites and fungi.

- Chemical: There are three principle types of chemical toxins found in foods: naturally occurring chemicals, e.g. cyanides in some root crops, and allergenic compounds in peanuts; toxins produced by micro-organisms, e.g. mycotoxins, and algal toxins; and chemicals added to the commodity by man to control an identified problem, e.g fungicides or insecticides.
- Physical: contaminants such as broken glass, metal fragments, insects or stones.

The probability that a hazard will occur is called a risk. The risk may take a value from zero to one depending on the degree of certainty that the hazard will be absent or that it will be present. After hazard identification, a hazard analysis must be conducted to understand the relative health risk to man or animal posed by the hazard. It is a way of organizing and analyzing the available scientific information on the nature and size of the health risk associated with the hazard. The risk may have to be assessed subjectively and simply classified as low, medium, or high. Only those hazards considered by the HACCP team to present an unacceptable risk of being present are taken forward to Stage 7, Principle 2.

Once a food safety hazard has been identified, then appropriate control measures should be considered. These are any action or activity that can be used to control the identified hazard, such that it is prevented, eliminated, or reduced to an acceptable level. The control measure may also include training of personnel for a particular operation, covered by GAP, GMP, and GHP.

Task 7 - Determine the critical control points (ccps) - (Principle 2).

Each step in the commodity flow diagram, within the scope of the HACCP study, should be taken in turn and the relevance of each identified hazard should be considered. It is also important to remember the stated scope of the HACCP analysis at this stage. The team must determine whether the hazard can occur at this step, and if so whether control measures exist. If the hazard can be controlled adequately, and is not best controlled at another step, ans is essential for food safety, then this step is a CCP for the specified hazard. A decision tree can be used to determine CCPs, and an example of the Codex decision tree is included in Appendix IV. However, the HACCP team's judgement, expertise and knowledge of the process are the major factors in establishing CCPs.

If a step is identified where a food safety hazard exists, but no adequate control measures can be put in place either at this step or subsequently, then the product is unsafe for human consumption. Production should cease until control measures are available and a CCP can be introduced.

Task 8 - Establish critical limits for each ccp - (Principle 3)

Critical limits must be specified and validated for each CCP. Criteria often used include measurements of temperature, time, moisture level, pH, water activity, and sensory parameters such as visual appearance. In the case of mycotoxins for example, they may include the moisture content or the temperature of the commodity. All critical limits, and the associated permissible tolerances, must be documented in the HACCP Plan Worksheet, and included as specifications in operating procedures and work instructions.

Task 9 - Establish a monitoring procedure - (Principle 4)

Monitoring is the mechanism for confirming that critical limits at each CCP are being met. The method chosen for monitoring must be sensitive and produce a rapid result so that trained operatives are able to detect any loss of control of the step. This is imperative so that corrective action can be taken as quickly as possible so that loss of product will be avoided or minimised.

Monitoring can be carried out by observation or by measurement, on samples taken in accordance with a statistically based sampling plan. Monitoring by visual observation is basic but gives rapid results, and can therefore be acted upon quickly. The most common measurements taken are time, temperature and moisture content.

Task 10 - Establish corrective action - (Principle 5)

If monitoring indicates that critical limits are not being met, thus demonstrating that the process is out of control, corrective action must be taken immediately. The corrective action should take into account the worst case scenario, but must also be based on the assessment of hazards, risk and severity, and on the final use of the product. Operatives responsible for monitoring CCPs should be familiar with and have received comprehensive training in how to effect a corrective action.

Corrective actions must ensure that the CCP has been brought back under control. They must also include appropriate disposition of any affected commodity or product. Whenever possible an alarm system should be introduced which will activate when monitoring indicates that the critical

limit is being approached. Corrective action can then be applied to pre-empt a deviation and prevent the need for any product disposition.

Task 11 - Verify the HACCP plan - (Principle 6)

Once the HACCP plan has been drawn up, and all of the CCPs have been validated, then the complete plan must be verified. Once the HACCP plan is in routine operation, it must be verified and reviewed at regular intervals. This should be a task of the person charged with the responsibility for that particular component of the commodity system The appropriateness of CCPs and control measures can thus be determined, and the extent and effectiveness of monitoring can be verified. Microbiological and/ or alternative chemical tests can be used to confirm that the plan is in control and the product is meeting customer specifications. A formal internal auditing plan of the system will also demonstrate an ongoing commitment to keep the HACCP plan up to date, as well as representing an essential verification activity.

Ways in which the system can be verified include:

- collecting samples for analysis by a method different from the monitoring procedure
- asking questions of staff , especially CCP monitors
- observing operations at CCPs
- formal audit by independent person

It is important to remember that the HACCP system is set up for a particular formulation of product handled and processed in a given way.

Task 12 – Keep record - (Principle 7)

Record keeping is an essential part of the HACCP process. It demonstrates that the correct procedures have been followed from the start to the end of the process, offering product traceability. It provides a record of compliance with the critical limits set, and can be used to identify problem areas. Furthermore, the documentation can be used by a company as evidence of 'Due Diligence Defence' as required, for instance, by the Food Safety Act 1990 (HMSO), in the UK.

Records that should be kept include: all processes and procedures linked to GMP, GHP, CCP monitoring, deviations, and corrective actions.

Documents should also include those that recorded the original HACCP study, e.g. hazard identification and selection of critical limits, but the bulk of the documentation will be records concerned with the monitoring of CCPs and corrective actions taken. Record keeping can be carried out in a number of ways, ranging from simple check-lists, to records and control charts. Manual and computer records are equally acceptable, but a documentation method should be designed that is appropriate for the size and nature of the enterprise. A template of a form to document product description and intended use is given in Appendix III, and a template of a HACCP Plan Worksheet is given in Appendix V. Examples of the use of these forms are provided in the case studies presented in Chapter 3.

APPLICATION OF HACCP TO MYCOTOXIN CONTROL

Once tasks 1 to 5 have been completed the following will be in place: a HACCP team, a Description and Intended Use table, and a verified Commodity Flow Diagram. This will provide information on a specific commodity from a unique source, and this information is required to complete the hazard analysis. See the case studies in Chapter 3 for examples of implementation, including that of stages 1 to 5.

Task 6 - Mycotoxin hazard analysis and identification of possible control measures

Hazard Analysis

a) Identification of mycotoxin hazard

For a given commodity system in a particular location, the HACCP team need to first consider which, if any, of the mycotoxins known to constitute a food safety hazard are likely to be present.

Over 300 mycotoxins are known, but only a relatively few of these are widely accepted as presenting a significant food or animal feed safety risk. These hazardous mycotoxins are listed in Tables 1 and 2 in Chapter 1. Of these only the following mycotoxins have regulatory limits set by one or more countries: the aflatoxins (including aflatoxin M_1), ochratoxin A, zearalenone, patulin, ergot alkaloids, and deoxynivalenol. Guideline limits exist for fumonisin B_1 and

regulatory limits are likely to be set in the near future. The regulatory limits are taken as the target levels and should be included in the Product Description table. Mycotoxin limits can also be set by the customer in specific contracts and it is possible that these may include mycotoxins not subject to regulatory limits.

The risk of a particular mycotoxin hazard should be estimated using well established data on the relative susceptibilities of commodities to given mycotoxins and the climatic conditions required for the mycotoxins to be produced. The EU has identified the following animal feed ingredients, and their products, as being highly susceptible to aflatoxin contamination: maize, groundnut cake, cottonseed cake, babassu, palm kernel cake and copra cake. The EU has also identified the following foodstuffs as highly susceptible to aflatoxin contamination: dried figs and other dried fruit, groundnuts, pistachios and other edible nuts and cereals. These commodities are specified in the respective EC regulations (1525/98 amending regulation 194/97). Maize grown in temperate climates would be less likely to be contaminated with aflatoxin, but could be contaminated with trichothecene mycotoxins or fumonisin B_1. Although published mycotoxin survey data exists for many commodities, it is important that surveillance studies are performed if mycotoxin data is lacking for a particular commodity, or for production in a particular climatic zone.

b) Identification of steps in the Commodity Flow Diagram (CFD) where mycotoxin contamination is most likely to occur

Once the mycotoxin hazard(s) has been identified, each step in the CFD must be considered in turn and the likelihood of mycotoxin contamination occurring must be assessed. Usually published scientific data will be available to act as a guide, but it may be necessary to commission a study to determine, or confirm that the correct steps have been identified. The situation may change from year to year, and season to season, so there will need to be an element of mycotoxin surveillance in the HACCP plan.

An important fact to establish is whether pre-harvest contamination with mycotoxins is likely or whether contamination occurs primarily post-harvest. Mycotoxins produced by *Fusarium* spp , such as fumonisin B_1 are invariably produced pre-harvest, but climatic conditions effect the degree of blight and the resultant level of mycotoxin contamination. Aflatoxins can be produced both pre-harvest and post-harvest and climatic conditions can have a significant bearing: drought

stress favours pre-harvest contamination, whereas post-harvest handling during the rainy season favours post-harvest aflatoxin contamination.

It is rarely possible to be certain that pre-harvest mycotoxin levels are below regulatory or target levels in the commodity system, so post-harvest mycotoxin control measures can often only prevent or reduce ADDITIONAL contamination, rather than prevent the hazard completely. Consequently it is often necessary to introduce a segregation step to remove any batches containing an unacceptable level of mycotoxin.

c) Possible Mycotoxin Control Measures

The most effective mycotoxin control measures is to dry the commodity such that the water activity (a_w) is too low to support mould growth and/ or prevent mycotoxin production. To prevent the growth of most moulds the a_w needs to be ≤ 0.70, which translates to a moisture content of approximately 14% for maize and 7.0% for groundnuts at 20°C (the corresponding moisture content decreases as the temperature increases). Each toxigenic mould has its own minimum water activity for growth and mycotoxin production and these translate into moisture contents for each commodity. These moisture contents are termed 'safe' and would be the critical limit for the control measure.

It is important to specify a target 'safe' moisture content with a maximum as well as an average value, e.g. 14% no part exceeding 15%. If only an average value is specified it may conceal a large range of moisture contents within the batch and the commodity would not be safe from mould growth and mycotoxin contamination. A drying process is required which dries evenly and the critical limits must be set bearing this in mind. Validation of such a CCP must involve moisture determination of multiple samples.

If the commodity is at an 'unsafe' moisture content for longer than 48 hours, then mould can grow and mycotoxins be produced. Hence limiting the time that the commodity spends in the 'unsafe' moisture content window to less than 48 hours is a control measure. This explains why timely sun-drying can sometimes be safer than delayed mechanical drying. Two days on a drying floor with occasional turning can often achieve the target 'safe' moisture content, whereas a back-log at the mechanical drier can result in the critical limit of 48 hours not being met.

Once produced, it is not usually possible to remove mycotoxins, other than by physical separation (grading) techniques. To apply this type of control measure, representative samples of batches of commodity are collected and tested for selected mycotoxins. Only those batches containing less than the critical limit of mycotoxin, as specified in official regulations, are accepted. For some commodities, such as blanched groundnuts, colour sorters may be effective in rejecting individual high-aflatoxin nuts and accumulating low-aflatoxin nuts, and may be classified as a control measure.

There are a few examples where effective chemical detoxification is possible, such as ammoniation of certain animal feed ingredients and refining of vegetable oils. These are control measures that would also be suitable for application at a critical control point for aflatoxin, but only for the specified commodities.

It is essential that GAP, GSP, and GMP pre-requisites are in place, and simply ensuring that this is the case can significantly reduce the risk of the mycotoxin hazard. Examples of procedures which fall within the scope of these pre-requisites include: irrigation, insect control, use of resistant varieties, and use of pallets in store.

Task 7 - Determine Critical Control Points (CCPs)

Determination of CCPs can be achieved using a well designed decision tree, if necessary, to supplement the knowledge and experience of the HACCP team (see Appendix IV). Each step in the CFD is considered in turn, and the questions answered in sequence. It should be noted that it is necessary to be able to answer Yes to Question 1 (Do preventative control measures exist?) before a CCP can be established. The Codex 1997 definition of a control measure is any action and activity that can be used to prevent or eliminate a food safety hazard, or reduce it to an acceptable level.

There are commodity systems, such as the production of apple juice (Case study 5), where control measures are possible at a number of steps, and each is capable of achieving a known percentage reduction in the level of mycotoxin. It is possible, therefore, to calculate the acceptable level of patulin at each step and perform validation. If the risk of the acceptable level of mycotoxin being exceeded is considered to be sufficiently low, then the HACCP team may determine each of the steps as CCPs.

Task 8 - Establish critical limits for each CCP

When the control measure is segregation based on mycotoxin analysis, then the critical limit will often be set at the acceptable level, which in turn will be set at, or below, the regulatory mycotoxin limit. Acceptable levels, and any associated critical limits, can sometimes be set higher than a regulatory limit, provided that a subsequent step can guarantee to attain the acceptable level of hazard in the final product.

For control measures that involve drying to a 'safe' moisture content, the parameter that will be measured, and for which critical limits will be set, will usually be parameters such as the temperature of the drier and the dwell time, e.g. for a continuous flow drier the critical limit for temperature could be 80 +/- 2°C and the critical limit for dwell time could be 20 +/- 1 minute.

Critical limits for chemical detoxification could be the temperature and pressure of the reaction vessel and the dwell time.

Task 9 - Establish a monitoring system for each CCP

The monitoring system must be a scheduled measurement, usually of a basic parameter such as temperature or time, to detect any deviation from the critical limits.

When segregation of acceptable and unacceptable batches is required in the agricultural system, for example at a secondary trader, then rapid testing procedures are needed to test incoming batches.

A number of semi-quantitative immunoaffinity rapid test kits are available which work to a stated target level, eg 5 or 20 µk/kg of the appropriate mycotoxin. Here the critical limit would normally be the presence or absence of a coloured derivative. More traditional mini-column and TLC dilution to extinction techniques can still be useful for segregation of batches at the factory gate, and for these the presence or absence of a blue fluorescent band or spot is the critical limit.

Task 10 - Establish a corrective action

There are two sorts of corrective action. The first is action to regain control. For instance if a critical limit for a moisture content is not attained, then the corrective action could be to check

the specification of the drier and effect repairs, or perhaps to increase the temperature setting or the dwell time. The second type of corrective action is to isolate the product produced whilst the CCP was out of control and amend the product disposition, by either discarding or down-grading it, or re-processing it if this is appropriate.

Task 11 - Establish verification procedures

At regular, specified, intervals the complete HACCP plan should be verified by checking that the levels of mycotoxin in the final product are within acceptable levels. If this is found not to be the case, then immediately trouble-shooting should be carried out to identify the step at which the hazard has become out of control. Critical limits may need to be amended, or a new control measure may need to be validated and introduced. Similarly, if a review of deviations and product dispositions indicated an unacceptable degree of control at a particular CCP, then revisions will need to be made.

Task 12 - Establish documentation and record keeping

Standard HACCP documentation and record keeping is appropriate, but the complexity of the records should reflect the sophistication of the step in the commodity system.

CONCLUSIONS

1. HACCP is a powerful tool with application to the control of mycotoxins in the commodity system.

2. Undertaking a HACCP study focuses the thinking of everyone involved with the product on the details of the process, and promotes a greater awareness of safety issues.

3. Implementation of a HACCP system is not an end in itself. The ongoing maintenance of the HACCP plan is where the benefit really lies.

Appendix I

Definition of terms

- **Based on Codex Alimentarius: HACCP System and Guidelines for its Application; Annex to CAC/RCP-1 (1969), Rev.3 (1997)**

Control (verb): To take all necessary actions to ensure and maintain compliance with criteria established in the HACCP plan.

Control (noun): The state wherein correct procedures are being followed and criteria are being met.

Control measure: Any action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Corrective action: Any action to be taken when the results of monitoring at the CCP indicate a loss of control.

Critical Control Point (CCP): A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Critical limit: A criterion which separates acceptability from unacceptability, when monitoring a critical control point.

Deviation: Failure to meet a critical limit.

Flow diagram: A systematic representation of the sequence of steps or operations used in the production or manufacture of a particular food item.

HACCP: A system that identifies, evaluates, and controls hazards which are significant for food safety.

HACCP plan: A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety in the segment of the food chain under consideration.

Hazard: A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Hazard analysis: The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan.

Monitor: The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control.

Step: A point, procedure, operation or stage in the food chain including raw materials, from primary production to final consumption.

Validation: Obtaining evidence that the elements of the HACCP plan are effective.

Verification: The application of methods, procedures, tests and other evaluations, in addition to monitoring to determine compliance with the HACCP plan.

- **Additional definitions**

Acceptable level: The level of a safety hazard which is considered to present an acceptable, low risk to the consumer. The acceptable level of the final product, sometimes referred to as a target level, should be stated in the product description and would normally be set at, or below, any regulatory limits. An acceptable level for a hazard at an intermediate step in the commodity flow diagram can be set higher than that of the final product, provided that the acceptable level in the final product will be achieved.

Commodity system: The complete system, including: all pre- and post- harvest activities such as growing, harvesting, drying, storage, processing, marketing, and preparation for home consumption.

Commodity flow diagram: A flow diagram which details and numbers each step in the commodity system.

Decision tree: A series of questions linked diagrammatically to be answered with Yes or No. The answers determine which path is followed and which decision this leads to.

Primary trader: The first trader in the marketing chain who typically buys small quantities of commodity direct from farmers and accumulates these for dispatch to a secondary trader. The primary trader will often carry out partial drying and temporary storage.

Product disposition: How the product is to be utilised. If a deviation occurs at a CCP, then part of the corrective action will be to amend the product disposition.

Real hazard: A hazard which has been identified as having a significant risk of being present.

Risk: May take a value from zero to one depending on the degree of certainty that the hazard will be absent or that it will be present

Safe molsture content: the moisture content at or below which toxigenic moulds cannot grow. Relates to a minimum water activity for mould growth and toxin production.

Secondary trader: A trader who typically buys commodity from a primary trader and (further) dries and stores it.

Target level: The acceptable level of a hazard in the final product, such as the regulatory level of mycotoxin in a product description.

Appendix II

Tasks involved in developing HACCP system

(Based on Codex Alimentarius, 1997)

Task 1.	Assemble an HACCP team
	⇓
Task 2.	Describe product
	⇓
Task 3.	Define essential characteristics of the product and intended use
	⇓
Task 4.	Construct a Commodity Flow Diagram
	⇓
Task 5.	On-site Confirmation of Flow Diagram
	⇓
Task 6.	List all Potential Hazards Conduct a Hazard Analysis Identify Control Measures
	⇓
Task 7.	Determine CCPs
	⇓
Task 8.	Establish Critical Limit for each CCP
	⇓
Task 9.	Establish a Monitoring System for each CCP
	⇓
Task 10.	Establish Corrective Action for deviations that may occur
	⇓
Task 11.	Establish Verification procedures
	⇓
Task 12.	Establish Documentation and Record Keeping

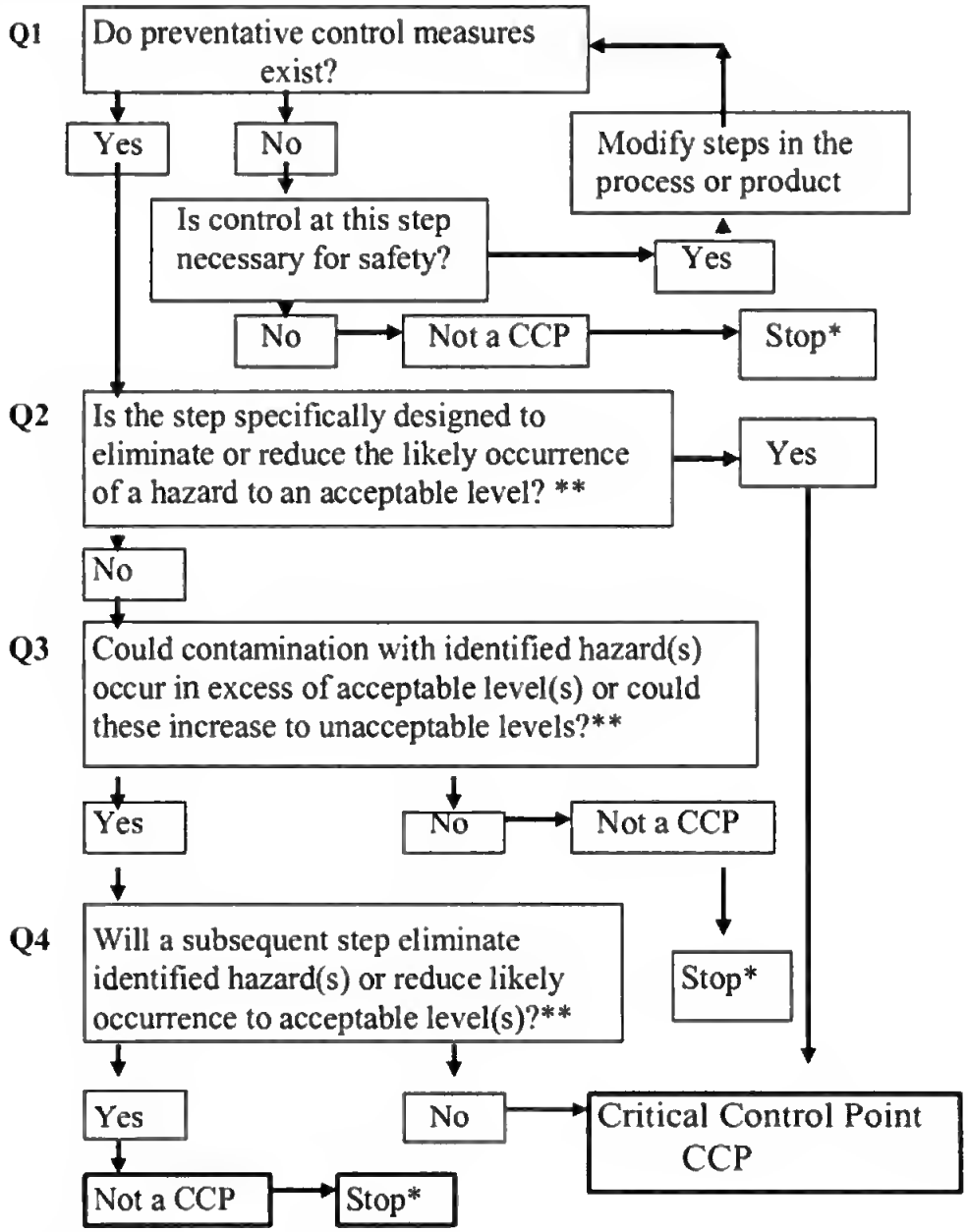
Example of Form – Description of a product and of its intended use

Name of product	
Full description of product including structure/variety, processing parameters, additive concentrations, storage instructions, pH/Aw/moisture levels, *and any mycotoxin target levels (regulatory or to customer specification).*	
Customer specification	
Conditions of storage and distribution	
Shelf Life	
Packaging	
Instructions on the label	
Target Consumer	
Recommendation for further processing required before consumption	
Intended use, e.g. will the end product be cooked before consumption?	

An example of decision tree to identify Critical Control Points (CCPs)

(The definition of control measure in Codex 1997 has been modified slightly for application to the production chain. The definition now includes activities used to prevent further contamination)

Answer questions in sequence



*Proceed to next hazard

**Acceptable levels needs to be defined

Appendix V

An Example of a HACCP Worksheet

1. **Describe Product**

2. **Commodity Flow Diagram**

3. **HACCP Analysis Plan**

Step	Hazard(s)	Control Measures	Control	Critical Limits	Monitoring Procedure	Corrective Actions	Records

4. **Verification**

Chapter 3

ILLUSTRATIVE EXAMPLES OF APPLICATION OF HACCP TO MYCOTOXIN CONTROL

The examples presented in this chapter aim to illustrate the application of HACCP to mycotoxin control. It must be emphasised that these HACCP plans are only examples and are for guidance only. Every HACCP plan must be developed following the 12 tasks, and applying the seven principles of HACCP. It is unlikely that any two HACCP plans will be identical, even for the same product.

The following examples are presented:

Example	Mycotoxin	Product	Region
1	Aflatoxin	Yellow maize kernels, for use in feed mills, both domestic and in importing countries. Target levels of aflatoxin $B_1 \leq 50$ or 20 µg/kg	South East Asia
2	Aflatoxin	Maize-based animal feed. Target level of aflatoxin B_1 dependent on the type of animal and its age: in the range of 5 to 50 µg/kg	South East Asia
3	Aflatoxin	Copra cake for animal feed. Target level of aflatoxin $B_1 = 20$ µg/kg	South East Asia
4	Aflatoxin	Peanut butter produced in a factory. Target level of aflatoxin $B_1 = 5$ µg/kg for local consumption, or the appropriate customer specification for exports	Southern Africa
5	Patulin	Apple juice. Target level of patulin ≤ 50 µg/L	South America
6	Aflatoxin	Pistachio nuts in shell, for export. Target level of total aflatoxin in range 4 to 20 µg/kg dependent on regulations in importing country, and whether further processing will take place	S. W. Asia

The target level of mycotoxin quoted is the regulatory limit in the country of origin, or the customer specification for exported products.
The examples have a strong aflatoxin bias, because this is the mycotoxin that is most widely regulated. Failure to meet the regulations will cause a food safety hazard in the country of origin and can cause loss of very important export markets, or be the major constraint in the promotion of an export market.

The format used for presenting the examples is a brief introduction followed by an outline of the 12 stages of HACCP, including a Description and Intended Use form, a Commodity Flow Diagram and a HACCP Plan worksheet.

Example 1: Yellow maize kernels - South East Asia

Introduction.

There are two crops possible in much of South East Asia: a major rainy season crop and a minor dry-season crop. The former is characterised by problematic post-harvest handling resulting in a high risk of mycotoxin contamination, whilst the latter is at low risk post-harvest, but more prone to pre-harvest contamination. There is often a surplus of maize and this is exported as an animal feed ingredient, thus generating valuable foreign exchange.

Exports of yellow maize produced in South East Asia, for use as animal feed, were under serious threat in the mid-1980s, due to difficulty in meeting regulatory limits for aflatoxin set by the major importing countries (e.g. = 20 µg/kg aflatoxin B_1 in the EU), and there was an urgent need for effective control measures. A project was carried out to address this problem and the findings (Nagler, M. J. et al 1987 and Jones, B. D. 1986) are used as a basis for Example 1.

Task 1 - The HACCP team

An appropriate HACCP team will be composed of: a HACCP specialist, a mycotoxicologist, a cereal grain specialist, a socio-economist, a mycologist, a drying engineer, and representatives of the maize industry in the public and private sectors.

Task 2 and 3 - Product Description and Intended Use

The product description and intended use is given in Table 3.

Tasks 4 and 5 - The Commodity Flow Diagram (CFD), Verified

The CFD will be established using information provided by members of the HACCP team, notably the cereal grain specialist, and representatives from the Department of Agriculture. It will be verified by visiting major maize production centres and interviewing farmers, traders, and silo and feed mill managers and observing their practices. An example of a typical commodity flow diagram is given in Figure 8.

Table 3. Product description and intended use of yellow maize kernels

Name of Product	Maize for animal feed
Description	Yellow maize kernels
Customer specification	Domestic: graded for Fair Average Quality Export: graded with aflatoxin limit of importer e.g. 20 µg/kg for the EU and Japan
Conditions of storage	Bulk in heaps or silos Bags in palleted stacks
Shelf Life	1 month if moisture content is <16% 3 months if moisture content is <14% 3 years if moisture content is <12%
Intended use	Animal feed, milled and usually mixed with other feed ingredients
Packaging	Bags, hessian, or polypropylene/ or bulk
Target Consumer	Feed mills, both domestic and in EU

Target level: ≤20 µg/kg aflatoxin B_1 for export to the EU and Japan.

≤50 µg/kg aflatoxin B_1 for domestic animal feed

Task 6: Mycotoxin hazard analysis and identification of possible control measures.

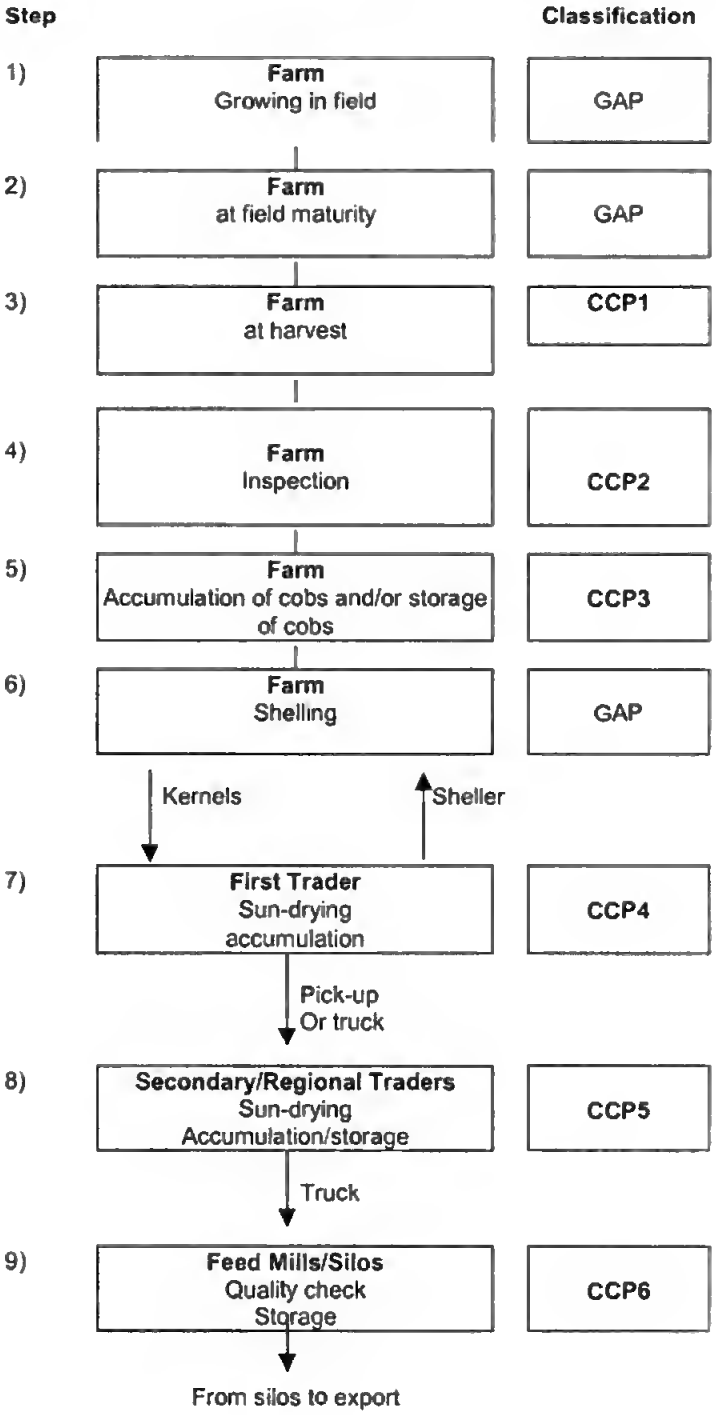
Hazard Analysis

a) Identification of mycotoxin hazard

Maize is very susceptible to aflatoxin contamination and this toxin has been classified as a human carcinogen and is the subject of regulation world-wide. Other mycotoxins which may be present include: zearalenone, one or more of the trichothecenes, and the fumonisins. Maize can be contaminated with more than one mycotoxin, and sometimes contains a cocktail of five or six. However, few countries have set regulatory limits for mycotoxins other than aflatoxin, so the HACCP team may well just concentrate on control of aflatoxin in the first instance.

In this example, aflatoxin is the only mycotoxin carried through to task 7.

Fig. 8. HACCP Flow-diagram: Yellow Maize in Southeast Asia



b) Identification of steps in the Commodity Flow Diagram (CFD) where mycotoxin contamination is most likely to occur.

Steps 1, 2, and 3: on farm, through growing and including harvest

Pre-harvest aflatoxin contamination is associated with drought stress and insect damage (Fortnum, B. A., 1986 and McMillian, W. W., 1986) during the final growing period. The dry-season crop is more prone to these conditions, but was found to be only moderately susceptible to significant levels of pre-harvest aflatoxin contamination. Surveillance studies, and field drying studies (Nagler, M J. et al, 1988) both indicated that levels of aflatoxin were very low at harvest in the rainy season crop, certainly in the locations under study and over the three year study period.

It is concluded that the risk of pre-harvest aflatoxin contamination is low, especially for maize produced in the rainy season.

Step 4: on-farm inspection of cobs.

Pre-harvest contamination with *Fusarium* mycotoxins will manifest as cobs showing obvious signs of ear rot. Incidence of ear rot was observed in maize produced in both the dry and the rainy season.

Step 5: on-farm accumulation of cobs and storage.

Surveillance studies and on-farm storage studies both indicated that aflatoxin B_1 levels rose to unacceptable levels, 60 to 90 µg/kg, when cobs were taken directly from the field and stored over a 1 to 6 month period, as was the usual practice.

It is concluded that aflatoxin contamination is very likely to occur at this step.

Step 6: shelling

No aflatoxin contamination is likely at this step. However if the percentage of broken grains produced was high, then this could pre-dispose the grain to contamination at a subsequent step.

Step 7: drying and accumulation at Primary Trader

Aflatoxin levels of freshly shelled maize rise very rapidly if the 'safe' moisture content is not attained within 48 hours. Surveys during the rainy season confirmed that aflatoxin contamination is extremely likely at this step.

Step 8: drying and storage at a secondary trader

Aflatoxin surveys showed that maize frequently became more contaminated with aflatoxin at this step.

Step 9: feed mills and export silos.

Feed mills and silos, even when buying maize at a 'safe' moisture content, buy maize of varying histories and a wide range of aflatoxin contents. Hence aflatoxin, produced at earlier steps in the CFD, can occur at this step.

Some silo owners have invested in high capacity mechanical driers and buy cheaper, 'wet' maize. Delays in introducing the maize to the driers, as witnessed by long queues of lorries and the use of 'holding silos' does introduce a high risk of aflatoxin contamination at this step.

c) **Possible Mycotoxin Control Measures**

The most effective aflatoxin control measure is drying to a moisture content that will not support the growth of toxigenic mould and the production of mycotoxins. For longer-term storage, further drying is required to prevent the growth of all moulds. A related control measure is the maintenance of a 'safe' moisture content.

Field-drying of the rainy season crop for up to 20 days was found to be very beneficial because moisture content of the cobs reduced from 35% at field maturity to less than 22%, allowing immediate shelling and reduced breakage. The lower moisture content made post-harvest drying easier, and **did not** result in any significant increase in aflatoxin contamination. Although this was true for this study, this may not always be found to be the case.

Drying and storage trials showed that aflatoxin contamination could also be prevented by two-stage drying of shelled maize. If the maize was initially dried to 16% (no part >16.5%) then it

could be stored safely for at least one week. This finding was consistent with the fact that *Aspergillus flavus* and *A. parasiticus* cannot grow and produce aflatoxin at a water activity a_w ≤0.82 at 25°C. Partial drying would allow a Primary Trader to part-dry maize kernels and then safely sell-on to a Secondary Trader who could complete the drying.

Segregation of acceptable from unacceptable batches of maize was another useful control measure. Although segregation by means of representative sampling and aflatoxin testing was employed as a control measure, it would preferably be used for verification only, once a high level of aflatoxin control had been attained at earlier steps in the CFD.

The use of mould resistant varieties, irrigation to prevent drought stress and use of insecticides or predators to control insects, are examples of GAP which can be effective in limiting pre-harvest mould and mycotoxin contamination.

It was considered to be GAP to shell cobs at an appropriate moisture content using a sheller that produced a low percentage of broken grains.

Tasks 7 to 10: Development of a HACCP Plan

A worksheet summarising the HACCP Plan for yellow maize kernels for animal feed is given in Table 4. The development of the plan at each step in the CFD is given below.

Step 1: Farm, growing in the field – GAP

Pre-harvest mould contamination can be alleviated by the use of relatively resistant varieties, e.g. varieties which have good sheaf cover and have cobs which droop early, allowing rain to run off easily. Insect, rodent and bird control can also be effective in reducing physical damage to the cob. Damage to cobs increases susceptibility to mould attack.

Step 2: Farm, at field maturity – GAP

Cobs have a very high moisture content, in the order of 35%, at field maturity in the rainy season. Harvesting cobs at high moisture makes it extremely difficult to dry to a moisture content low enough for safe storage, or low enough for shelling, without suffering mould damage and mycotoxin contamination. It is advisable not to harvest at field maturity, unless damage is likely to be higher by delaying harvesting, e.g. high incidence of pest damage.

Step 3: Farm, at harvest – CCP1

Although this step was not identified as a step where aflatoxin was very likely to normally occur, it was found that a control measure introduced at this step could reduce the likelihood of subsequent mould contamination, to an acceptable level. Field-dried maize could be shelled directly, with a low percentage of breakage, and then dried relatively easily to a 'safe' moisture content. Step 3 is therefore determined to be a CCP with field-drying being the control measure for rainy season maize. The critical limit for this CCP is ≤22% moisture content and monitoring is by farmer testing. With training, traditional techniques such as biting kernels, or weighing freshly shelled kernels in the hand, can be used to assess the moisture content.

In this study, the increased risk of pre-harvest aflatoxin contamination during field-drying was heavily outweighed by the resulting reduced post-harvest contamination. This may not always be the case in other locations, and under different climatic conditions. Hence the more generalised CCP would be to harvest at an appropriate time.

Step 4: Inspection on farm – CCP2

This step was identified as a CCP with segregation of obviously mouldy cobs as the control measure. This CCP with reduce the percentage of mouldy cobs to an acceptable level, and hence reduce the levels of any mycotoxins produced pre-harvest. It with also reduce the likelihood of biodeterioration and subsequent mycotoxin production, which can occur when mouldy cobs are stored. An appropriate critical limit could be rejection of cobs showing mould damage over >10% of the surface. This CCP is best monitored by trained harvesters.

Step 5: Accumulation and storage of cobs on-farm – CCP3.

This step is identified as a CCP with two alternative control measures. The first is to dry the cobs to ≤16% m.c. within two days of harvest, prior to storage. However, if this is not possible, or storage is not desired, then the cobs should be shelled within one week of harvest, and preferably within two days. These control measures will prevent any significant subsequent production of aflatoxin. Critical limits, other than the final moisture content, can be set in terms of sun-drying time which will produce the required final moisture content.

It is advisable to avoid the use of polypropylene bags until maize is dried to 14% m.c.

For medium-term farm storage (1 to 6 months), Good Storage Practice (GSP) is required to prevent mould contamination. Examples of such practices include: a sound roof, good ventilation, raised floor, insect and pest control.

Step 6: Shelling - GAP

Minimising the percentage of broken kernels produced during shelling is considered Good agricultural Practice. Broken kernels allow easier infection by aflatoxin producing moulds and this can lead to higher levels of aflatoxin contamination if subsequent CCPs move out of control. Hence, if GAP is not applied correctly at this step, the effect will manifest itself as a more extreme product disposition if corrective actions are required at a subsequent CCP.

To minimise breakage during shelling, the moisture content of maize cobs must be in the correct range for the mechanical sheller used. If the maize cobs are wet, say at a moisture content in excess
of 20%, they will be too soft for many shellers and damage will be high. Conversely, maize cobs which are very dry, say at a moisture content below 15%, may be brittle.

Step 7: Primary Trader – CCP4.

Drying freshly shelled kernels to ≤16% m.c. within 48 hours is the control measure adopted to establish this step as a CCP. However, Primary Traders currently rely on sun-drying, which is least reliable when it is needed most, during the rainy season. Critical limits are set for sun-drying to attain some measure of control, but to attain the required degree of control, mechanical drying is required. Unfortunately this is rarely financially viable at the Primary Trader step, but can be at Step 8. Primary Traders therefore need to move maize swiftly on to Secondary Traders during bad weather.

Primary Traders usually carry out short-term storage of maize, to allow them to accumulate sufficient maize to trade with the Secondary Trader. Good Storage Practice is required in order to prevent re-wetting, e.g. a store with a sound roof and the use of pallets to prevent water being soaked up from the ground.

Step 8: Secondary Trader – CCP5.

This step is determined to be a CCP with drying to a moisture content of 14% (no part >15%), prior to storage as the control measure.

Some Secondary Traders have mechanical driers that are used to supplement sun-drying and these are essential when sun-drying is impossible.

It is important that Good Storage Practice is in place. As well as measures to prevent re-wetting, insect and rodent control will be required if mycotoxins are to be prevented during medium and long-term storage.

Step 9: Feed mills and export silos –CCP6.

If the CCPs at the previous steps could be implemented fully, then verification rather than a CCP will be appropriate at this step. However, it will take time to fully and satisfactorily implement this HACCP Plan in the commercial sector, so a segregating CCP is appropriate. The critical limit for the CCP is set at the required target level of aflatoxin and monitoring will be by means of representative sampling and aflatoxin analysis using semi-quantitative testing.

Those export silos with a policy of buying wet maize and using mechanical driers should match procurement with drying capacity. Delayed drying will result in mould contamination, heating, and rapid aflatoxin production.

Good storage practice is necessary at this step to prevent re-wetting and damage due to pests.

Task 11: Establish Verification Procedures

Validation procedures will be established for each of the CCPs and overall verification will be provided by the fully quantitative aflatoxin results on representative samples of the batches incoming to domestic feed mills, or on representative pre-loading samples of maize destined for export.

The control measures were validated on the 10 tonne scale, in replicates of 10, at each of two locations in major production areas. Maize produced according to this HACCP plan averaged less than 5 μg/kg in both locations, whilst maize produced without these control measures in place in the commercial sector averaged just under 200 μg/kg.

The HACCP Plan will be audited quarterly and amended as necessary.

Task 12: Establish documentation and record keeping

The HACCP Plan will be fully documented, with appropriate record keeping at each step.

Table 4. HACCP Plan Worksheet: Aflatoxin in yellow maize kernels for animal feed.

Process Step	Description of hazard	Possible Control Measures
1 and 2 Farm growing	AFLATOXIN CONTAMINATION *(low risk in rainy-season crop, higher risk in dry-season crop)*	
	Mould	*Resistant varieties, eg early drooper*
	insects	*Insecticide, predators*
3 Harvesting	*Mould*	**Field dry* up to 20 days in rainy season** (to facilitate post-harvest control)
4 Farm inspection	*Mould*	**Discard mouldy cobs**
5 Farm Accumulation	*Mould (post-harvest contamination) (low risk in dry-season crop, high risk in rainy-season crop)*	***Minimize time cobs are at >16% m.c.***
Storage	*Mould/ aflatoxin*	**Dry maize cobs to 'safe' a_w of 0.82 before entering store.** Prevent re-wetting in store maximize ventilation
	Insects	Insecticide, inert dust or organic
6 Farm shelling	*Mould*	Minimise broken kernels by shelling at <22% m.c.
7 first trader short-term grain storage	*Mould* *Very high risk in rainy-season, low risk in dry-season*	***Dry kernels to moisture content for safe storage for up to 1 week ie m.c. <=16% no part >16.5%, within 48 hours*** FINANCIAL INCENTIVE to farmers for freshly harvested maize at <= 22% m.c. Improved store design to increase ventilation Do not use poly-propylene bags, use hessian

8 Secondary trader. larger quantities & longer storage	*Mould* *high risk in rainy-season, very low risk in dry-season*	***Dry maize to moisture content safe for medium-term storage, 14%, no part >15%*** FINANCIAL INCENTIVE required to First Trader for low-moisture maize at <16%
9 Feed mills/ Silos huge quantities long-term	*Aflatoxin* *Insects*	**Improved grading: reject or down-grade maize containing excessive aflatoxin or excessive moisture** Minimise delays on trucks awaiting testing and unloading **(avoid heating)** Fumigation or modified atmosphere
Inter-step Transport Pick-up/ Truck		Avoid poly-propylene bags when maize is >14% m.c. Minimize time on truck Dry maize adequately, and evenly, before transportation Use tarpaulins when raining, remove when weather is fine

* Leave un-harvested in the field after field-maturity
** GSP = Good Storage Practice

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Example 2: Maize-based Animal Feed - South East Asia

Introduction

This HACCP plan follows on directly from Example 1 and considers utilisation of the yellow maize kernels in a feed mill in Southeast Asia. The mill will usually offer contracts to Secondary Traders for the supply of maize of a specified quality, but may also buy from visiting traders, especially if stocks are low.

Task 1 - The HACCP team

An appropriate HACCP team will include: the plant manager or his deputy, the quality control manager, the procurement manager, the senior engineer, and the quality control laboratory manager.

Tasks 2 and 3. - Table 5: Product Description and Intended Use

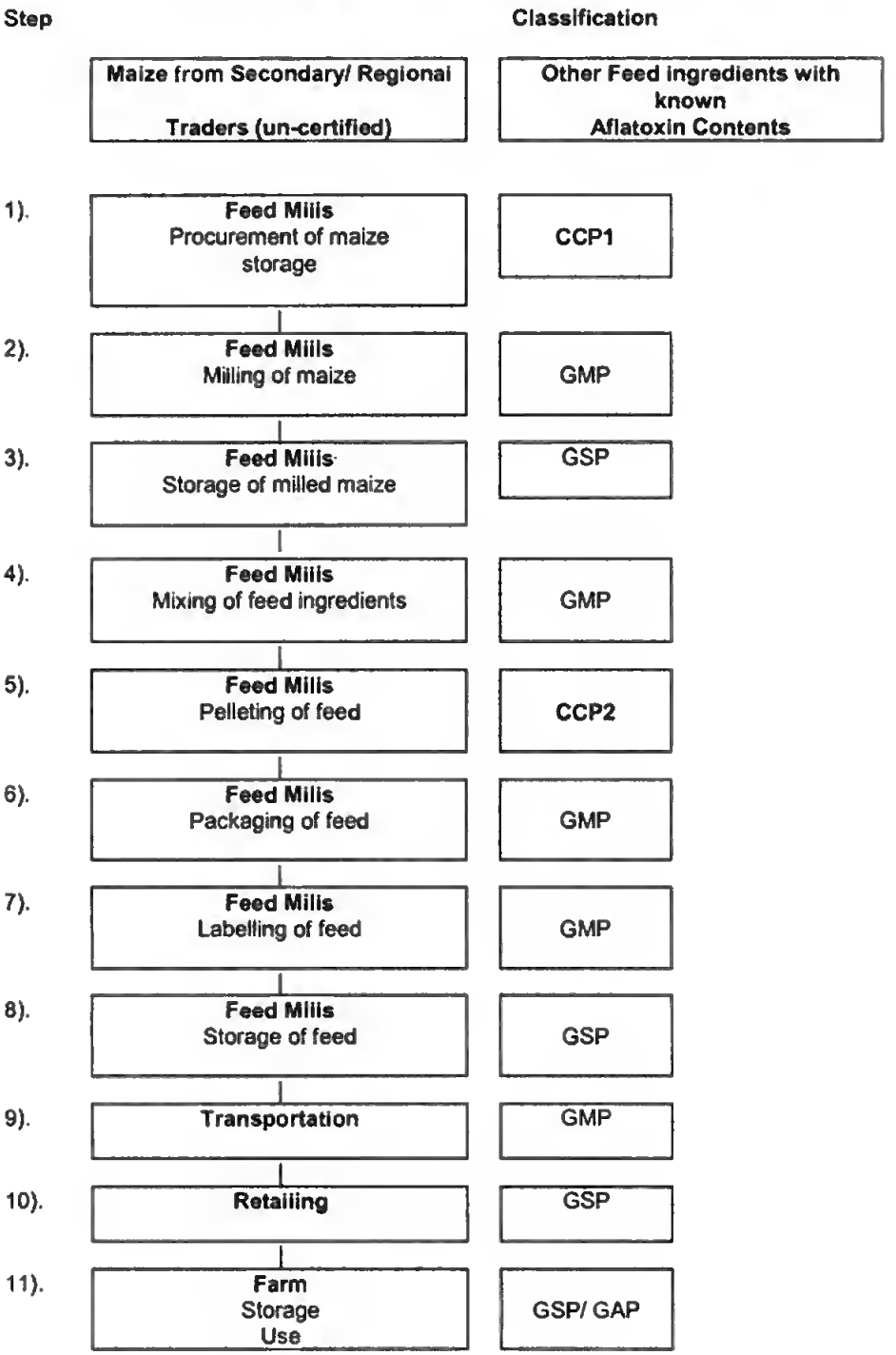
Name of Produet	Maize for animal feed
Description	Milled maize or maize-based mixed animal feed for specified animals and specified ages
Customer specifieation	Nutritionally balaneed safe feed with mycotoxins within regulatory limits for the specified feed, typically in the range of 5 to 50 µg/kg aflatoxin B_1
Conditions of storage	Bags in pelleted stacks
Shelf Life	3 months when pelleted and m.c. <13%
Intended use	Animal feed
Packaging	Multi-layered bags, often waxed or polythene coated to reduce moisture transfer
Target Consumer	Specified animals of specified age

Target limit = in the range 5 to 50 µg/kg, depending on animal

Tasks 4 and 5 - The Commodity Flow Diagram (CFD), Verified

The CFD was established and verified and an outline summary is shown in Figure. 9.

Fig. 9. HACCP Flow-diagram: Maize-based feed in Southeast Asia



Tasks 6: Mycotoxin hazard analysis and identification of possible control measures.

Hazard Analysis

a) Identification of mycotoxin hazard

Maize is susceptible to a number of mycotoxins including: zearalenone, one or more of the trichothecenes, ochratoxin A and fumonisin B_1, in addition to the aflatoxins. Maize can be contaminated with more than one mycotoxin, and sometimes contains a cocktail of five or six. These mycotoxins can significantly reduce animal production, and there is a possibility of carry-over to the human food chain. Few countries have set regulatory limits for mycotoxins other than aflatoxin, so for this example the HACCP team has identified aflatoxin as the major mycotoxin hazard.

b) Identification of steps in the Commodity Flow Diagram (CFD) where mycotoxin contamination is most likely to occur.

Step 1: Procurement and storage

Most of the aflatoxin, and indeed other mycotoxins, found in feeds are usually present in the incoming raw materials.

Step 2: Milling

Aflatoxin contamination is unlikely at this stage, provided that normal cleaning is carried out, as would be expected under Good Manufacturing Practice (GMP)

Step 3: Storage of milled maize.

Aflatoxin contamination is unlikely at this step provided that moisture content is controlled at Step1.

Step 4: Mixing of feed Ingredients.

This step and needs to be performed correctly to ensure that the aflatoxin content of the mixed-feed is within the target level.

Step 5: Pelleting

Aflatoxin contamination can occur at this step as a result of adding too much water during pelleting, or perhaps after pelleting, in order to maximise the moisture content.

Steps 6: Packaging

Aflatoxin contamination is unlikely at this step, and indeed correct packaging can provide protection from subsequent contamination.

Step 7: Labelling

Correct labelling is very important to ensure safety of the feed.

Step 8: Storage of mixed feed

Feed mills rarely store mixed feed for long, and indeed feed is usually despatched and used by farmers within two or three weeks. There is little risk of aflatoxin contamination at this step.

Step 9: Transportation

Animal feed is usually distributed by truck, but sometimes it may be shipped between islands. There is little risk of aflatoxin contamination during transportation.

Step 10: Retailing

Aflatoxin contamination of feed is unlikely at this step.

Step 11: Farm storage and use

Poor on-farm storage and feeding practices can result in aflatoxin contamination of feed.

c) Possible Mycotoxin Control Measures

The most important control measure is to procure maize, and other feed ingredients, which have only a low, acceptable risk of containing unacceptable levels of mycotoxin. This can either be achieved by buying maize with reliable certification, or by segregating acceptable from unacceptable batches on procurement.

Prevention of mycotoxin in the feed mill is best controlled by ensuring that the procured maize is at a 'safe' moisture content on procurement, and that the moisture content remains too low to support mould growth during all subsequent steps.

Selection of appropriate formulations to produce feed within the aflatoxin specification for each type of feed is essential.

Tasks 7 to 10: Development of a HACCP Plan

A worksheet summarising the HACCP Plan for the production of maize-based mixed feeds is given in Table 6. The development of the plan at each step in the CFD is given below.

Step 1: Procurement and storage, CCP1

It is essential to either purchase maize that has reliable mycotoxin certification, or to segregate acceptable from unacceptable batches on procurement. This control measure will prevent unacceptable levels of mycotoxin entering the feed mill.

The critical limit will be 50 µg/kg aflatoxin B_1 in this example, but critical limits for other mycotoxins can also be set if required. The critical limit is monitored by collecting representative samples, ideally in the form of a pre-loading sample collected from the suppliers warehouse. Failing this, representative samples taken from individual trucks, or groups of trucks can be collected for testing. The samples are then analysed by a rapid mini-column or test kit method, and only those batches containing an acceptable level of mycotoxin are accepted.

It is also very important to procure feed ingredients which have a moisture content at or below the 'safe' level corresponding to an water activity (a_w) of 0.70.. This control measure will prevent aflatoxin, and other mycotoxins, being produced within the feed mill.

The critical limit for maize is an average moisture content of 14%, but it is most important that no bags should contain >15% moisture, so spot samples are required as well as representative samples. The critical limit is monitored by measuring the moisture content of representative and 'spot' samples from each batch, using a regularly calibrated moisture meter.

Aflatoxin levels would not be expected to rise during storage, provided that the moisture content requirements mentioned above have been met. Normal Good Storage Practice (GSP), such as storage on pallets, a clean store, first in first out, and a sound roof will suffice. When mills store raw materials for extended periods, to ensure supply or to buy when prices are low, then insect and rodent control become important. However, these procedures are still covered by GSP.

Step 2: Milling of maize, GMP

Cleaning to prevent a build-up of dust within the mill, which might become a source of mould and contamination, is required.

If the mill conditions maize by adding water prior to milling, then this process would need to be controlled, and the step would become a CCP.

Step 3: Storage of milled maize, GSP

It is uncommon to store milled maize for longer than a few days. Good storage practice will prevent significant increase in moisture content and subsequent mould contamination.

Step 4: Mixing of feed ingredients, GMP

Selection of appropriate batches of feed ingredient, and dietary formulations, to produce feed within the aflatoxin specification for each type of feed is essential. A good estimate of the level of aflatoxin in each of the ingredients of the formulation must be known and the level of aflatoxin in the mixed feed can then be calculated. The batches of ingredient used have to be carefully selected, especially for low-aflatoxin feeds. Sometimes it might be necessary to change the formulation to meet aflatoxin criteria.

This step might be considered to be a CCP, but is covered by GMP if the controls at Step 1 are in place.

Step 5: Pelleting of feed, CCP2

Moisture is added to feed in the form of dry steam during the pelleting process. It is critical to cool the pellets to ambient temperature using sufficient aeration to effect drying to a 'safe' moisture content.

A critical limit of moisture content is set at 13% for pellets just prior to packaging. This critical limit is monitored by the collection of a representative sample from each batch. The moisture content of each of these samples is then measured using an appropriate, calibrated moisture meter.

The addition of dry steam at 110°C during pelleting will sterilise the feed. Mould spores present in the feed will be killed and this will reduce the likelihood of any subsequent mould contamination.

Step 6: Packaging of feed, GMP

Correct packaging, such as use of moisture barrier bags, will prevent re-wetting of the feed and subsequent mycotoxin contamination.

Step 7: Labelling of feed, GMP

Correct labelling is very important, for instance the mis-labelling of a bag containing beef cattle feed (at, say, 49 µg/kg aflatoxin B_1) as a dairy feed (target level ≤5 µg/kg) would have serious implications. However, control of this procedure is covered by GMP.

Include certification that the feed meets aflatoxin regulations.

Step 8: Storage of feed, GSP

Feed is rarely stored for more than a few days at a feed mill, and no special storage practices are required.

Step 9: Transportation, GMP

Water barrier packaging will protect the feed during transportation.

Step 10: Retailing, GSP

The retailer should not stock feed that has passed the sell-by date and should not store opened or damaged bags of feed.

Step 11: Farm storage and use, GSP/ GAP

Farm storage must be adequate to prevent wetting of the feed.

Farmers should not use feed that has passed its use-by date. Feed dispensers must be cleaned daily to prevent the growth of mould on left-over feed.

Task 11: Establish verification procedures

Validation procedures are required for each of the CCPs and overall verification of the HACCP Plan is provided by aflatoxin results on representative samples of batches of feed leaving the feed mill.

Complaints from farmers or traders would be logged and followed-up, especially if a pattern developed which was consistent with an outbreak of aflatoxicosis. This could indicate that the HACCP plan has failed and needs to be amended.

The HACCP Plan would be audited quarterly and amended as necessary.

Task 12: Establish documentation and record keeping

The HACCP Plan is fully documented, and records kept of the CCP monitoring data, deviations and corrective actions.

Table 6. HACCP Plan Worksheet - Maize-based animal feed – South East Asia

Process Step	Description of hazard	Possible Control Measures	Control Step?	Critical Limits	Monitoring Procedures	Corrective Actions	Records
1 Feed mill Incoming maize	Aflatoxin contamination	Segregate and accept only batches of maize containing acceptable levels of aflatoxin Limit moisture content to prevent subsequent aflatoxin contamination	CCP1	>50 ppb aflatoxin B1 <14% mc, no part >15%	Sampling and rapid aflatoxin testing on truck-loads or batches is preferred Moisture meter, at least 10 point samples	Reject batch Change supplier if level of rejects is unacceptable Dry or reject	Laboratory reports Laboratory reports
2 Feed mill Milling	Aflatoxin contamination	Clean mill to prevent mouldy deposits and carry-over	GMP				
3 Feed mill Storage of milled maize	Aflatoxin contamination	Good Storage Practices Minimizing storage time	GSP				
4 Feed mill Introduction of other feed ingredients/mixing	Aflatoxin contamination	Formulate feed and selected batches to meet target levels of aflatoxin in specified feed	GMP				
5 Feed mill Pelleting	Aflatoxin contamination	Control moisture content of pellets by cooling with adequate aeration	CCP2	Mc < or = 13%	Moisture meter	Additional drying	Mill records
6 Feed mill Packaging	Aflatoxin contamination	Use of appropnate packaging, e.g. multi-layered bag with plastic liner for hygroscopic feeds	GMP				
7 Feed mill Labelling	Aflatoxin contamination	Ensure correct labelling Certify feed as low-aflatoxin	GMP				
8 Feed mill Storage	Aflatoxin contamination	Good storage practice, minimizing storage time	GSP				
9 Transportation	Aflatoxin contamination	Prevent re-wetting	GSP/GMP				
10 Retailing	Aflatoxin contamination	Minimize storage time	GSP				
11 Storage Use	Aflatoxin contamination	Buy aflatoxin-certified feed Minimize storage time Clean feed dispensers	GAP GSP GAP				

Example 3: Copra cake and meal - Southeast Asia

Introduction.

Coconut oil is produced by extracting oil from copra, which is dried coconut flesh. When the oil is expelled mechanically the residue is called copra cake and if this is then solvent extracted to increase the yield of oil, the product is called copra meal. These copra by-products are valuable protein sources in animal feeds, especially dairy feed. In the early 1990s the European Union tightened aflatoxin B_1 regulations on dairy feed to 5 µg/kg and also reduced the limit for aflatoxin B_1 in copra by-product to 20 µg/kg. This action put the vitally important export market for copra by-product in jeopardy. Loss of the market, worth US$ 80 million to one country in Southeast Asia alone, would have made many of the oil mills non-viable and would have caused great hardship to millions of coconut farmers.

A HACCP-type approach was used to try to save the European market by increasing very substantially the number of batches meeting the new regulations and by raising confidence in the product in Europe. The findings of the associated research (Andanar, W., 1991 & Anon., 1993) have been used as a basis for this example.

Task 1 - The HACCP team

An appropriate HACCP team will be composed of: a HACCP specialist, a mycotoxicologist, an oilseeds specialist, a socio-economist, a mycologist, a drying engineer, and representatives of the coconut oil industry from both the public and private sectors.

Tasks 2 and 3 - Product Description and Intended Use, Verified

This information is given in Table 7.

Tasks 4 and 5 - The Commodity Flow Diagram (CFD), Verified

The CFD will be established using information provided by the HACCP team, and will be verified by visiting major copra production centres and oil mills and interviewing key players and observing their practices. An example of a typical commodity flow diagram is given in Figure 10.

Table 7. Product description and intended use for Copra Cake and Meai

Name of Product	Copra cake or copra meal
Description	Coconut flesh residue after oii expelling (cake) or after additional . soivent extraction (meal)
Customer specification	≤12% m.c., pelletised <20 ppb aflatoxin B_1
Conditions of storage	Ambient temperature in processors' warehouse (25-35°C).
Shelf Life	Up to 12 months at ≤12% m.c.
Intended use	Animai feed component for incorporation into poultry and ruminant feed, particularly dairy feed
Packaging	Buik, hold of ship
Target Consumer	Feed compounders in the EC

Target levei: ≤20 µg/kg aflatoxin B_1

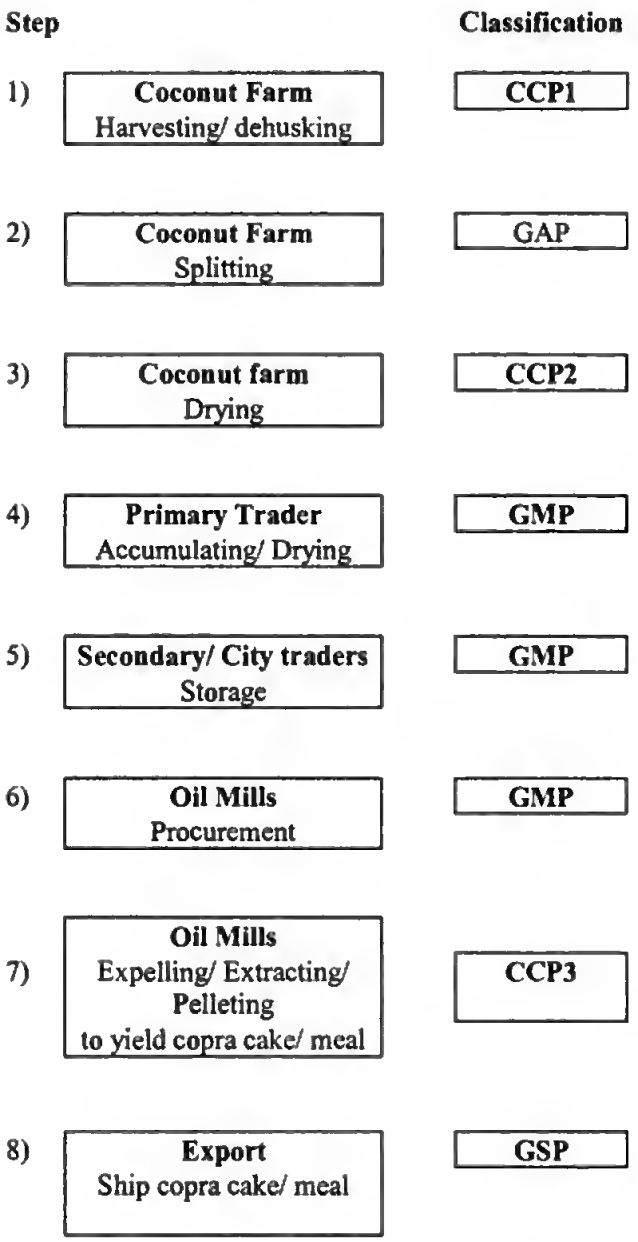
Task 6: Mycotoxin hazard analysis and identificatiou of possible coutrol measures

Hazard Anaiysis

a) Identification of mycotoxin hazard

Aflatoxin is the only mycotoxin hazard for which regulatory limits have been set to protect animal health and production, and also to ensure that levels of aflatoxin M_1 (a metaboiite of aflatoxin B_1) in milk are within a very strict limit of 0.05 µg/Litre.

Figure. 10. Verified commodity flow diagram for Example 3.



b) Identification of steps in the Commodity Flow Diagram (CFD) where mycotoxin contamination is most likely to occur

Surveillance studies and controlled experiments were undertaken to determine at which steps aflatoxin contamination was most likely to occur. It was found that aflatoxin was produced within 10 days of splitting the coconut, when the coconut meat was at a water activity of >0.82, and aflatoxin producing moulds could grow. This situation occurred during the drying process, at steps 3 (on the farm) and/ or step 4 (at the Primary Trader). Levels of aflatoxin were always zero prior to splitting, provided that the nut was sound. If the nut had been split prematurely, during harvest or de-husking, then these nuts could be contaminated prior to drying. Reject nuts from the desiccated coconut industry were often prematurely split and represented a special case.

Risk of aflatoxin contamination was low in subsequent steps, apart from Step 7 where pellets could be produced at too high a moisture and be susceptible to mould and mycotoxin contamination.

c) Possible Mycotoxin Control Measures

Drying uniformly to a 'safe' moisture content within 48 hours of splitting the nut was found to be by far the most important control measure.

Surveillance studies strongly indicated that traditional smoke drying was correlated with low-aflatoxin copra.

Average levels of aflatoxin in sun-dried copra were found to be very high. This was mainly because four or five full days are required to attain the 'safe' moisture content and farmers usually only dried for 2 or 3 days. Lengthening the drying time was not a complete solution because the copra could easily still be in the 'unsafe' moisture content window for >48 hours and contamination could occur during drying. Also this scenario assumes perfect sun-drying weather. If drying is slowed by cloudy weather, or interrupted by rain, then there is a very high risk that high levels of aflatoxin B_1 will result. Therefore, discouraging sun-drying was considered a control measure.

An incentive was required for farmers and traders to produce low-aflatoxin copra. This was provided in an amended Government grading scheme which introduced grading on the basis of

percentage yellow-green mould. It also increased the premiums for dry copra, so that it made it worthwhile to dry to a 'safe' moisture content.

Tasks 7 to 10: Development of a HACCP Plan

A spreadsheet summarising the HACCP Plan for copra by-product is given in Table 8. The development of the plan at each step in the CFD is given below.

Step 1: Farm, harvesting and dehusking – CCP1

This step was classified as a critical control point with a control measure to eliminate the use of nuts found to be split during harvesting and dehusking. This CCP would eliminate any aflatoxin already present.

The critical limit will be set at zero cracked nuts and it will be monitored by trained harvesters or de-huskers. The CCP can be validated by determining the aflatoxin content of batches of accepted nuts.

Step 2: Farm, splitting nuts – GAP

Coconuts are split into halves, or sometimes smaller pieces, immediately prior to drying. It is advisable to ensure that the coconut meat is protected from contact with soil, which is a rich source of inoculum. This is considered GAP.

Step 3: Farm, drying – CCP2

This was classified as a CCP with drying to a safe moisture content within 48 hours being the control measure. This CCP will prevent the growth of mould and production of aflatoxin.

Controlled drying and storage trials showed conclusively that direct smoke drying protected copra from aflatoxin contamination: copra only needed to be dried to a moisture content of ≤16% to store safely, whereas hot-air dried or sun-dried copra needed to be dried uniformly to ≤12% to prevent aflatoxin contamination. These moisture parameters are achieved by setting critical limits on the drying time. Different types of dryer will require different drying times, and different copra turning schedules, to achieve the safe moisture content. For example, smoke

drying will take 24 hours with turning of copra cups every 8 hours, whereas a commonly used hot-air drier will take 30 hours, with a change in bed position every 10 hours.

The critical limits are monitored by timing the drying period and the scheduled turning or moving of the copra on the drying bed. Validation of the CCP will be achieved by measuring the moisture content of the product.

Step4: Primary trader, procurement and drying – GMP/GSP

A national grading system, which provides a premium price for Grade 1 copra showing <1% yellow-green mould (characteristic of *Aspergillus flavus* or *A. parasiticus* which produce aflatoxin) and meeting a 12% moisture limit, was introduced. It is considered GMP for Primary traders to purchase grade 1 copra, and keep it separate from lower grade copra.

Primary traders currently also purchase lower-grade copra, at moisture contents up to 18%. They purchase this at a lower price and then dry to 12% moisture. This practice often leaves copra at an unsafe moisture content for longer than 48 hours and results in a greatly increased risk of mould and aflatoxin contamination.

Primary traders store dried copra for a short period, whilst they accumulate sufficient to sell to a Secondary trader. Good storage practice will ensure that the copra remains dry.

Step 5: Secondary traders, procurement and storage – GMP/ GSP

Procurement of Grade 1 copra is also GMP at this step. The Grade 1 copra must be kept separate from other grades and marketed as low-aflatoxin copra.

Good storage practice, such as palleted storage in a store with good ventilation and a sound roof, will prevent re-wetting and subsequent contamination with mould and aflatoxin (Head, S. W., 1999).

Lower grade copra will dry in store and successive colonies of moulds will grow until the safe moisture content of 12% is reached. The copra no longer looks mouldy at this stage, and there is certainly no sign of yellow-green mould. However, such copra retains pitting of the surface associated with penetrating moulds and can be identified in this way.

Step 6: Oil mills, procurement – GMP

Procurement of Grade 1 copra is essential to produce copra by-products containing acceptable levels of aflatoxin, and this is considered to be GMP. It should be noted that the mould classification now includes copra that is pitted.

Oil millers tend to buy and store large stocks of copra. Provided that the copra is at, or below, a moisture content of 12%, then aflatoxin will not be produced with good storage practice in place. It is important to have adequate aeration, however, because 'hot spots' can develop and these can even result in spontaneous combustion.

Step 7: Oil mill, expelling/ extracting/ pelleting – CCP3

No aflatoxin control measures are necessary during expelling of oil and solvent extraction. In fact the high temperatures present during expelling will sterilise the copra meal, destroying mould spores and hence reducing the risk of subsequent contamination.

The pelleting process within Step 7 was classified as a CCP, with a critical limit of 12% moisture in the cooled pellets. Insufficient cooling, or insufficient aeration of the pellets will result in an unacceptable moisture content. For a given process, the critical limits will be the dwell time in the cooling tower and the air-flow. These critical limits will be monitored by timing the dwell time and measuring the air-flow. The CCP will be validated by regularly determining the moisture content of the cooled pellets.

Pelleted copra by-product is stored either in bags or in bulk until shipment is possible. Good storage practice will prevent any subsequent aflatoxin contamination.

Step 8: Shipment – GMP/ GSP

No increase in aflatoxin contamination is likely during shipping, provided that the copra by-product is loaded at a moisture content of ≤ 12%, and does not suffer sea-water damage. Practices, such as opening holds during fine weather will help reduce further any risk of mould damage.

A number of shipments of copra by-product were closely monitored, and no increase in aflatoxin levels were found.

Task 11: Establish verification procedures

Validation procedures will be established for each of the CCPs, as indicated above, and overall verification will be provided by the fully quantitative aflatoxin results on the pre-loading samples, taken immediately prior to export.

The HACCP Plan will be audited quarterly and amended as necessary.

Task 12: Establish documentation and record keeping

The HACCP Plan will be fully documented, including appropriate records at the farmer and Primary trader steps.

Table 8. HACCP Plan Worksheet, Copra By-Product in Southeast Asia

Process Step	Description of hazard	Possible Control Measures	Control Step?	Critical Limits	Monitoring Procedures	Corrective Actions	Records
1 Farm Harvest/ de-husking	Mould	Select sound nuts only	CCP1	No visible crack	Inspection of nuts	Discard splits	Farmer
2 Farm Splitting		Avoid soil contamination	GAP	No visible soil on flesh	Inspection of nuts	Wash off soil	Farmer
3 Farm Drying	Mould	Dry to 'safe' m.c. within 48 hrs of splitting nut a) Smoke drying to <=16% m.c. no part >17% or b) Hot-air drying to <=12% m.c. no part >13%	 CCP2a CCP2b	Into drier within 12 hours of splitting nuts Dry for >=24 hours Turn copra every 8 hours Dry for >=30 hours Change position on bed every 10 hours	Time period before drying Time drying Scheduled turning Time drying Scheduled moving	Discard nuts left too long Further drying/discard Turn asap after Further drying/discard Move asap after	Farmer Farmer Farmer Farmer
4 Primary Trader Procurement/ Drying	Mould	National grading system Procure Grade 1 copra		GMP/ GSP			
5 Secondary traders Procurement/ Storage	Mould	National grading system Procure Grade 1 copra		GMP/ GSP			
6 Oil Mill Procurement/		National grading system Procure Grade 1 copra		GMP/ GSP			
7 Oil Mill expelling/ pelleting	AFLATOXIN CONTAMINATION	Control moisture of pelleted product during cooling and aeration	CCP3	Final moisture content <=12%	Moisture determination of representative samples	Further cooling/ aeration to effect drying	Mill records
8 Shipment Export	AFLATOXIN CONTAMINATION	Prevent re-wetting during shipment		GMP/ GSP			

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Example 4: Commercially produced peanut butter, Southern Africa

Introduction

The eommodity system represented here is similar to those systems frequently found in sub-Saharan Afriea, where the small-seale produetion of groundnuts is praetised in eombination with the commercial manufacture of peanut butter.

Small scale eommercial or peasant farmers' crops are usually short-season, low-input eultivars (cvs) which are intended to be grown within the rainy season, without irrigation. Short season evs are often more resistant to aflatoxin produetion than long-season cvs.

Peanut butter is produced from groundnut kernels by roasting, grinding and mixing processes. Emulsifiers are added to ensure that the oil released by grinding remains in suspension. Groundnut processing systems are very eomplex, involving a variety of manufacturing lines producing differently specified products; a single product line is considered in the eurrent example.

Task 1 - The HACCP team

The HACCP team will include: HACCP specialist, production manager, factory quality assurance manager, mycologist, mycotoxicologist, commodity specialist, socio-economist, agronomist and representatives of trading and export sectors

Tasks 2 and 3 - Product description and intended use

Table 9 shows the produet description and intended use.

Tasks 4 and 5 - The Commodity Flow Diagram (CFD), Verified

The CFD was established and verified and is summarised in Figure 11

Table 9. Product Description and Intended Use of End Product

Name of product	Peanut butter
Description	Peanut butter containing emulsifiers and additives to give Type A and Type B produets
Conditions of storage	Ambient, in consumers' home
Shelf life	Type A: 5 months Type B: 3 months
Intended use	Type A: consumed fresh Type B: added to cooking
Packaging	Glass jar with sealed lid
Customer specification	Type A: Smooth, flowing paste with no off-flavours Type B: Stiff, non-flowing paste with aflatoxin specification of <20 µg/kg total aflatoxin (US and local specifieation)
Target consumer	Whole family, especially infants

Task 6 - Mycotoxin hazard analysis and identification of possible control measures

Hazard Analysis

a) Identification of mycotoxin hazard

Aflatoxin is the only mycotoxin hazard for whieh the region has regulatory limits for groundnuts and, consequently, it is the only mycotoxin considered. It is also the key myeotoxin associated with groundnuts.

b) Identification of steps in the Commodity Flow Diagram (CFD) where myeotoxin contamination is most likely to oeeur

Step 1: On farm, pre-harvest

Pre-harvest contamination with aflatoxin-produeing *Aspergillus* moulds is associated with drought stress and insect damage, both of which are difficult to control without access to

Fig. 11. HACCP Flow-diagram: Peanut Butter in Southern Africa

Step			Classification
1)	**Farm** Growing in field	Salt Emulsifiers	GAP
2)	**Farm** Harvest - cut tap roots by hand or machine and hand-lift haulm		GAP
3)	**Farm** Windrow - invert		**CCP1**
4)	**Farm** Sun dry on sheet or on racks		**CCP2**
5)	**Farm** Sort, during removal of pods from haulms		CCP3
6)	**Farm** Store bagged, inshell		GAP
7)	**Trader/processor** Accumulation		GMP
8)	**Trader/processor** Shell, Grade on size.		GMP
9)	**Factory** Aflatoxin test		**CCP4**
10)	**Factory** Roast		GMP
11)	**Factory** Hand-sorting		GMP
12)	**Factory** Grind		
13)	**Factory** Pack		GMP

irrigation and expensive insecticides. Insect damage provides an entry point for mould propagules, which are often carried by the insect itself. Drought stress can also cause pod splitting in the soil, leaving the kernels exposed to the soil microflora.

Steps 2: On-farm harvesting

Additional contamination with *Aspergillus* moulds is unlikely during the harvesting process.

Steps 3 & 4: On-farm, windrow and sun-drying

Contamination by *Aspergillus* moulds will occur at these steps if a 'safe' moisture level is not achieved within a short period of time.

Step 5: On-farm, removal of pods from haulm

Additional contamination with *Aspergillus* moulds is unlikely during the removal of the pods from the groundnut haulm.

Steps 6-8: Farm to trader or processor

No aflatoxin contamination should occur here, if the commodity is properly stored and handled.

Step 9: At factory, aflatoxin testing of incoming batches of groundut kernels

There is no risk of additional contamination at this step.

Step 10: At factory, roasting

There is no risk of additional contamination at this step.

Step 11: At factory, hand-sorting

There is no risk of additional contamination at this step.

Steps 12 & 13: At factory, grinding and packing

There is no risk of additional contamination at this step.

c) Possible Mycotoxin Control Measures (Table 10)

If pre-harvest contamination is to be avoided, the most effective control measures will involve those procedures associated with GAP, which prevent mould contamination. Such control measures will include the prevention of drought stress and insect damage, the use of fungal-resistant varieties (if available), the use of fertilisers and the control of weeds. The use of biological control agents such as atoxigenic strains of *Aspergillus flavus* has been piloted in the USA and Australia, but has not, as yet, been widely adopted as a fully acceptable practice.

Immediately after harvest, it is essential that the groundnuts are dried to a 'safe' moisture level ($a_w \leq 0.82$) as rapidly as possible. Artificial drying procedures are not generally available in southern Africa, where sun-drying is normally performed on the haulm, by a combination of windrow drying followed by drying on a flat surface.

A further control measure is the segregation of contaminated nuts during harvesting, drying and handpicking. The ultimate segregation procedure is the inspection of individual batches of groundnuts, by sampling and aflatoxin analysis, and the rejection of excessively contaminated batches.

Tasks 7-10 - Development of a HACCP Plan,

A worksheet summarising the HACCP Plan for peanut butter is given in Table 10

Step 1: On farm, pre-harvest - GAP

GAP will prevent pre-harvest mould contamination, although the effects of inclement weather (drought stress, prolonged rains) can be extremely difficult, if not impossible, to control.

Step 2: On-farm harvesting - GAP

Again, GAP will prevent pre-harvest mould contamination. The selection of the appropriate harvesting time is important so that the groundnuts are harvested immediately they reach maturity. It is also important that the pods are not damaged during harvesting, in order to maintain a protective environment for the groundnut kernels.

Steps 3 & 4: On-farm, windrow and sun-drying – CCP1 and CCP2

These steps are both CCPs, since mould and aflatoxin contamination will rapidly occur if the groundnuts are not dried to a safe moisture level ($a_w \leq 0.82$) as rapidly as possible. The precise combination of moisture level and maximum permitted drying period will vary with groundnut variety and agroclimatic zone, and will need to be determined using local knowledge. It is envisaged that the preliminary windrow drying should achieve a moisture level of ≤12%, whereas the second drying phase, on a flat surface, should attain a ≤7% moisture level. However, mould and aflatoxin contamination can still occur if the safe moisture levels are not attained sufficiently rapidly.

Step 5: On-farm, sorting during removal of pods from haulm – CCP3

The control measure is the rejection of pods which are discoloured, mouldy and/or damaged whilst the pods are being removed from the haulm. The critical limit will be determined by the maximum percentage of unacceptable pods which, typically, can be removed during the harvesting process. For the purposes of this example it is assumed that 95 per cent of unacceptable pods could reasonably be expected to be removed during harvesting.

Steps 6-8: Farm to trader or processor - GAP

The careful storage and handling of both the pods and the kernels is classified as GAP and GMP. However, it is essential that the groundnuts are maintained in a clean, dry and undamaged state during these steps, if contamination is to be avoided.

Step 9: At factory, aflatoxin testing of incoming batches of groundnut kernels

Step 9 is a CCP which segregates those batches of groundnuts which contain an unacceptable level of aflatoxin B_1. The aflatoxin content of every batch of groundnut kernels is determined by selecting a representative sample of 20kg, at least, and analysing for aflatoxin using a simple, semi-quantitative test kit. (More sophisticated analytical methods such as high performance liquid chromatography, HPLC, may be used, of course, if available.) The critical limit will be an acceptable level of aflatoxin B_1 which, after the implementation of steps 10 and 11, will allow the target level of
20 μg/kg aflatoxin B_1 to be attained. In this example, the HACCP team considered that the critical limit should be 30 μg/kg aflatoxin B_1.

In a situation where it is felt that pre-harvest contamination is under control, or is not a significant problem, Step 9 may eventually be utilised as a component of the verification procedure.

Step 10: At factory, roasting - GMP

The roasting of the groundnut kernels under appropriate conditions is considered as GMP. However, the roasting process can cause a 20-30% reduction in aflatoxin contamination, depending upon the operating conditions utilised.

Step 11: At factory, hand-sorting - GMP

The primary aim of the hand-sorting process is the removal of burnt kernels, which will have a detrimental effect on the quality of the peanut butter. However, Step 11 also provides a final opportunity to remove obviously shrivelled and/or damaged kernels before the grinding process; and, potentially, to simultaneously reduce the level of aflatoxin in the final product. The HACCP team was uncertain whether hand-sorting *after* roasting would have a significant effect on the aflatoxin level and, consequently, did not define step 11 as a CCP. However, this step would become a CCP if subsequent studies clearly demonstrated the efficacy of the hand-sorting process as a means of removing aflatoxin.

Steps 12 & 13: At factory, grinding and packing - GMP

The quality of the grinding and packing procedures is controlled by GMP. The grinding procedure, used in the conversion of kernels to peanut butter, will effect the distribution of aflatoxin in the end-product, but will not change the overall level of contamination.

Stage 11: Establish verification procedures

Verification procedures will be established for each of the CCPs, and the HACCP plan will be regularly audited, and amended as necessary

Stage 12: Establish documentation and record keeping

The HACCP plan will be fully documented, and appropriate records kept at each CCP.

Table 10. HACCP Plan Worksheet: Peanut butter produced in southern Africa

Process Step	Description of hazard	Possible Control Measures	Control	Critical Limits	Monitoring Procedures	Corrective Actions	Records
1 Farm Growing in field	MOULD CONTAMINATION	Control drought stress and insect damage Use of fertilisers Use of resistant varieties	GAP				
2 Farm: Harvest: cut tap roots & hand lift haulms	MOULD CONTAMINATION	Harvest when nuts mature Remove & burn diseased plants Protect haulms from rain Avoid damage to pods	GAP				
3 Farm. Windrow drying	MOULD CONTAMINATION	Dry to safe moisture level (e g ≤12%)	**CCP1**	Safe moisture level & maximum drying period to be determined locally	Timing of drying period	Remove mouldy nuts	Farmers records
4 Farm: Sun dry on sheet or on racks	MOULD CONTAMINATION	Dry to safe moisture level (e g ≤7%)	**CCP2**	Safe moisture level & maximum drying period to be determined locally	By observing physical characteristic of nut eg crackling noise when pod is shaken	Remove mouldy nuts	Farmers records
5 Farm: Sorting, during manual removal of pods from haulm	MOULD CONTAMINATION	Avoid damage to pods Discard unacceptable pods	**CCP3**	≤ 5% unacceptable pods remain	Visual inspection of pods during removal from haulm	Resort pods and remove remaining unacceptable pods	Framers recors

6 Farm: Store, in-shell, in bags	MOULD CONTAMINATION	Keep pods clean & dry	GAP				
7 Trader/Processor: Accumulation	MOULD CONTAMINATION	Keep pods clean & dry	GAP				
8 Trader/Processor: Shell & size grade	MOULD CONTAMINATION	Keep pods & kernels clean & dry	GAP				
9 Factory: Inspection	AFLATOXIN CONTAMINATION	Collect 20kg representative sample from incoming batches and analyse for aflatoxin	CCP4	Aflatoxin $B_1 \leq 30$ µg/kg	Aflatoxin testing using rapid kits	Reject batches not meeting aflatoxin specification	Factory records
10 Factory: Roast	AFLATOXIN CONTAMINATION	Roast at appropriate temp.	GMP				
11 Factory: Hand- sorting	AFLATOXIN CONTAMINATION	Remove shrivelled & burnt kernels by hand picking	GMP				
12 Factory: Grind	AFLATOXIN CONTAMINATION	Ensure cleanliness of equipment	GMP				
13 Factory: Pack	AFLATOXIN CONTAMINATION	Use clean, airtight packing	GMP				

Example 5: Apple juice (Apple drink) - South America

Introduction

There is a significant risk that levels of patulin in apple juice produced in South America will exceed a 50 µg/kg target level. A survey carried out on apple juice in Chile (Canas, P. 1996) found a 28% incidence of samples of apple juice and apple concentrate exceeding this limit.

Apple juice produced in Latin America is different to that produced in Europe in that it has added sucrose and water, as well as the preservative sodium metabisulphite.

Task 1 - The HACCP team

An appropriate HACCP team will be composed of: a HACCP consultant, a mycotoxicologist, a mycologist, a quality assurance manager at the processing plant, a process engineer, representatives of the farmers and the Department of Agriculture, and a scientific secretary. A specialist in the area of fruit juice production and legislative matters will be consulted as and when necessary.

Tasks 2 and 3. - Product Description and Intended Use, Verified.

This information is given in Table 11.

Tasks 4 and 5 - The Commodity Flow Diagram (CFD), Verified (Figure 12)

The CFD will be prepared and verified by a series of visits to the orchards and processing plant. A typical CFD is presented in Figure 12.

Task 6 - Mycotoxin hazard analysis and identification of control measures

a) Identification of mycotoxin hazard

Patulin was the only mycotoxin hazard identified in this product. A number of European countries including Switzerland, Belgium, Austria and France have a 50 µg/Litre limit. The lowest limit is 30 µg/kg, in Romania.

Table 11. Description and Intended Use of End Product

Name of Product	Apple juice
Description	13° Brix apple juice with added sugar, preservative (sodium metabisulphite) and water. Filtered through 5 micron filter, pasteurised at 90°C for 2 minutes
Conditions of storage	Bulk tank at reduced temperature until processed. Ambient temperature when processed
Shelf Life	Six month at ambient. Chilled and consumed within 4 days once opened
Intended use	Consumed without further heating.
Packaging	Glass bottle or tetrapack - 1 litre
Customer specification	Acid level important to product taste. Within microbiological and mycotoxin guidelines
Target Consumer	Local consumption and export. All age groups

b) Identification of steps in the CFD where mycotoxin contamination is most likely to occur.

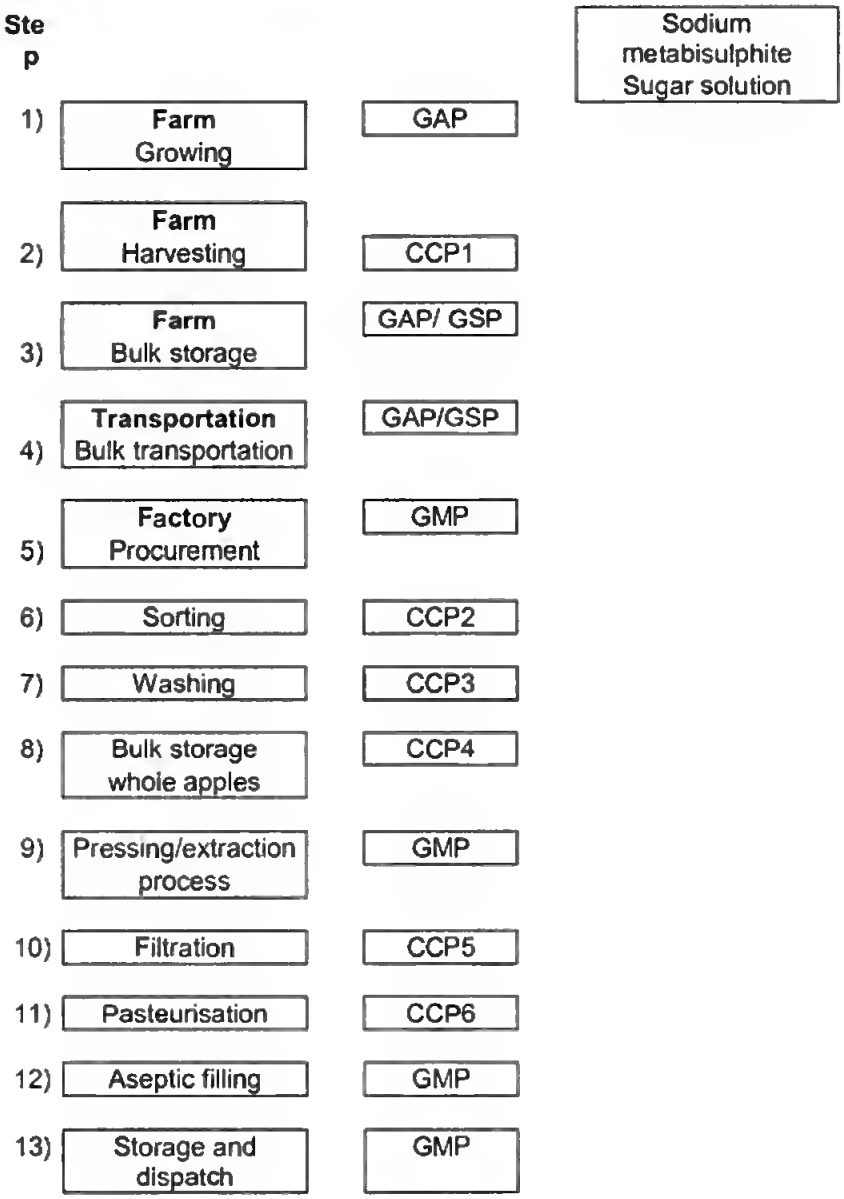
Each step in the CFD will be considered in turn.

Patulin contamintion is likely to be produced in the orchard during growing (Step 1) and during bulk storage (Step 3). There is little risk of further contamination during transportation, but damage to apples at this stage can increase the risk of subsequent contamination.

At the factory, patulin contamination is most likely to increase during storage at Step 8.

There is likely to be patulin contamination present in the apples, or the resultant apple juice, at every step in the commodity chain. Hence it is important to both minimise contamination, and reduce levels of contamination to the acceptable level.

Fig. 12. HACCP Process Flow-diagram: Apple juice



c) Possible Patulin Control Measures

Contamination of the juice can be prevented at steps where rotten or rotting apples can be rejected from the process, either in the orchard when the fruit is harvested, or during sorting in the factory.

Post-harvest patulin contamination can be eliminated, or significantly reduced, by storage at <10°C, and by minimising storage times.

Washing, and in particularly pressure spraying, has been shown to be effective in removing patulin from apples.

Patulin can also be removed from apple juice by filtration, when patulin bound to solid particles of apple flesh are removed.

Inactivation of *Penicillium expansum* spores during pasteurisation at Step 11 will reduce the risk of patulin production in the finished juice.

Tasks 7 to 10: Development of a HACCP Plan

A spreadsheet summarising the HACCP plan for patulin in apple juice is given in Table 12. The development of the plan at each step in the CFD is given below.

Step 1: Farm, growing in the orchard - GAP

Growth of the mould *Penicillium expansum*, and subsequent patulin contamination, can occur pre-harvest, where it is associated with damaged and over-ripe fruit. Good Agricultural Practice (GAP) will minimise insect and bird damage.

Step 2: Farm, at harvest – CCP1

The control measure at this step is to efficiently reject rotten and damaged apples during harvesting. Rotten apples are much more likely to contain high levels of patulin than sound looking apples. In one study (Sydenham, E. W., 1995), as much as 70% of patulin present in a batch of over-ripe apples was removed by sorting and removing visually mouldy apples. Application of this control measure at Step 2 is considered a CCP because it will reduce mould contamination to an acceptable level.

The effect of this CCP on levels of patulin in the system should not be considered in

Table 12. HACCP Plan Worksheet, Apple Juice, S. America

Process Step	Description of hazard	Control Measures	Control	Critical limits	Monitoring Procedures	Corrective actions	Records
1 Orchard growing	Mould / Pests	Minimise damage caused by birds and insects	GAP				
2 Orchard Harvest	Mould	Remove mouldy and damaged apples Avoid trash and soil contamination	CCP1 GAP	<1% visibly mouldy apples	Visual observation	Discard	Farm records
3 Farm Cooling and bulk storage	Mould	Reduce risk factors Handling and storage at <10°C to minimise mould growth	GAP/ GHP[2]	All staff to be trained	Check training records Automated readout	Discard Adjust temperature Check monit. system Inspect fruit	Farm records
4 Transportation	Mould	Avoid damage and mould contamination	GAP / GHP				
5 Factory Procurement	Mould	Inspect and reject low-grade apples with >10% mould apples	GMP	<10% damaged fruit	Quality check on representative sample	Reject batch	Factory records
6 Factory Sorting	Mould / Patulin	Remove mouldy apples	CCP2	<1% visibly mouldy apples	Visual observation of samples	Discard or re-sort Adjust inspection procedure	Operator log % reject

[2] GHP = Good Horticultural Practice

Process Step	Description of hazard	Control Measures	Control	Critical limits	Monitoring Procedures	Corrective actions	Records
7 Factory Washing	Mould / Patulin	Leach patulin from apples. Remove rotten parts of fruit containing patulin with pressure spraying	CCP3	Critical soaking time and pressure of spray system	Time of soaking step; regular check of water spray pressure	Repeat the washing step	Factory records
8 Factory Bulk storage	Mould / Patulin	Temperature control to <10°C in store, and minimise time in store	CCP4	<10°C temperature or <48 hours in store	Thermometer reading Storage time	Check monitoring system Inspect fruit	Factory records
9 Factory Pressing/extract.	Mould / Patulin	Cleaning Batch segregation	GMP GMP				
10 Factory Filtration	Patulin Mould	Remove patulin in particles	CCP5	Size and quality of particles remaining	Laboratory test	Un-block/replace filter Re-filter juice	Factory records
11 Factory Pasteurisation	Mould	Destroy *Penicillium expansum* spores	CCP6	Correct time/Temp.	Automated readout	Re-pasteurise?	Factory records
12 Factory Aseptic filling			GMP				
13 Factory Storage & dispat			GMP				

isolation. The HACCP team will consider the cumulative effects of subsequent CCPs and will judge whether levels of patulin in the final product are likely to exceed acceptable levels. The HACCP team will also consider the fact that removal of mouldy apples at this step will reduce the risk of subsequent patulin production, especially during on-farm storage. There is a subsequent sorting step at Step 6, so it could be argued that sorting is not required here. However, there are strong arguments to support sorting at both steps. Failure to sort at Step 1 will result in greatly increased patulin production at Steps 3, and unnecessary transportation of rotten fruit. There is little doubt that application of this sorting control measure at Step 1 is important for the production of apple juice containing acceptable levels of patulin.

The critical limit for this CCP will relate to the percentage of visibly mouldy apples remaining after sorting, and will be determined by the sorting efficiency which can reasonably be expected at this stage. For this example, the HACCP team considered that 99 per cent of mouldy apples should be removed at this step. The procedure will be monitored by trained supervisors and verified by a grading check on representative samples.

Step 3: Farm, bulk storage - GAP

Application of GAP and GSP is necessary to minimise rotting of fruit and subsequent patulin production during bulk storage. Storage of sound apples is important and the length of storage should be minimised, unless refrigerated storage facilities are used.

Step 4: Transportation – GAP

There is little risk of patulin contamination during short duration journeys, but any physical damage sustained during transportation, including loading and unloading, will predispose the fruit to subsequent mould attack and possible patulin contamination. The correct handling of fruit is therefore required.

Step 5: Factory procurement – GMP

Procurement of batches of low-grade apples, with a high percentage of damaged and rotten fruit, are to be avoided. It could be argued that, with a sorting step to follow, the procurement of low-grade apples would be permissible. However, batches containing >10% rotten fruit, say, will be extremely difficult to sort manually, and the levels of patulin likely to be present will make it difficult to attain an acceptable level of patulin in the finished product.

Step 6: Factory sorting – CCP2

The control measure is sorting to remove visibly mouldy apples. This CCP will reduce the level of mould to an acceptable level, and make a major contribution towards achieving an acceptable level of patulin in the final product. Sorting will both remove mouldy apples missed during sorting at Step 2, and remove apples that have subsequently become mouldy at Steps 3 and 4.

As for Step 1, the critical limit for this CCP will be the acceptable percentage of mouldy apples remaining after the sorting procedure, and monitoring will be by use of a trained supervisor.

Step 7: Factory, washing – CCP3

The control measure is washing the apples using high-pressure water spraying to remove rotten apple flesh, and patulin, from the fruit. Studies (Acar, J., 1998, & Sydenham, E.W., 1995) have shown that washing in this way can remove more than half of the patulin present in the fruit. The critical limits for this CCP will be related to the pressure of the sprays and the duration of the washing step. The water pressure will be monitored using pressure gauges and the washing step will be timed.

Patulin levels will be reduced at this step, but spores will be suspended in the water. This inoculum will increase the risk of mould growth during bulk storage.

Step 8: Bulk storage of whole apples – CCP4

The control measure is to prevent mould growth and patulin production by storing at reduced temperature. If refrigerated storage is not available, then storage time must be minimised. The critical limits are either a storage temperature of <=10°C or a maximum storage time at ambient temperature of 48 hours. These critical limits for temperature are monitored by means of a calibrated thermometer, preferably with a continuous chart read-out, and the storage period is monitored by a timing device.

Step 9: Pressing/ extraction process – GMP

Good Manufacturing Practice will ensure that the presses are cleaned regularly to prevent a build-up of mouldy apple waste which could be a source of patulin contamination.

Step 10: Filtration – CCP5

The control measure is the removal of fine, patulin-rich particles held in suspension in the crude juice. Research has shown (Acar, J., 1998) that a significant reduction in levels of patulin can be achieved using filtration. Conventional clarification by means of a rotary vacuum precoat filter resulted in a 39% reduction in levels of patulin, and ultrafiltration resulted in a 25% reduction. Critical limits are set for the size and quantity of particles remaining in the apple juice after filtration. These critical limits are monitored by microscopic examination of samples of apple juice.

Step 11: Pasteurisation – CCP

This step is a CCP for the control of bacterial hazards. However, it can also be considered as a CCP for control of the patulin hazard since pasteurisation will destroy spores of *Penicillium expansum*, and therefore prevent any subsequent mould growth, and patulin production, in submerged culture in the apple juice.

Although patulin levels are unlikely to be reduced significantly during pasteurisation, mould spores will be destroyed and the risk of patulin being produced subsequently in the apple juice will be reduced.

Step 12: Asceptic packaging process - GMP

Following pasteurisation, it is important to prevent the re-introduction of micro-organisms, including mould spores, during packaging. These procedures are covered by GMP.

Packaging is selected which will protect the juice from contamination by micro-organisms, e.g. tetra packs, or glass bottles with air-tight seals for the lid.

Step 13: Storage and dispatch - GMP

No subsequent contamination with patulin is likely.

Tasks 11: Establish verification procedures

The HACCP plan will be audited quarterly, and amended as necessary.

Tasks 12: Establish documentation and record keeping

The HACCP Plan will be fully documented, and appropriate records kept at each CCP.

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Example 6: Pistachio nuts in West Asia

Introduction

Pistachio nuts in this Region are grown commercially in Afghanistan, Iran, Iraq and Turkey. In the first three countries the nuts are usually dehulled very soon after harvest and the nuts in shell are then stored and processed (fast lane). In Turkey, however, the nuts are stored in-hull, sometimes for many months, or even for years (slow lane). Early de-hulling has the advantage of avoiding staining of the shell, but has the disadvantage of exposing the split nuts at an early stage to *Aspergillus flavus* and *A. parasiticus* spores which have the potential to produce aflatoxin.

The pistachio nut is the fruit stone of *Pistacia vera*. Each fruit has a single stone which consists of a kernel covered by a testa and enclosed in a shell. The shell itself is enclosed in a protective hull. One month or more before maturity the shell usually partially splits within the hull. The hull should remain intact, but sometimes it also splits naturally prior to harvest and these 'early splits' and 'growth-splits' are particularly susceptible to aflatoxin contamination. Early splitting allows invasion by insects, particularly the navel orangeworm [*Amyelios transitella* (Walker)] and insect-damaged nuts are associated with a high risk of aflatoxin contamination.

Different varieties of pistachio trees are grown in the Region. In Iran, Iraq and Afghanistan varieties are grown which tend to have large nuts with hulls which are relatively prone to early splitting, although climatic factors also have a bearing on this. In Turkey the pistachio varieties tend to yield smaller nuts with greener kernels and these have hulls which are not very susceptible to early splitting.

De-hulling can either be carried out using a wet-process or a dry process. The former is used by large-scale factories and some medium-scale factories, whereas the latter process is carried out mostly in cottage industries.

Collecting a representative sample of pistachio nuts for aflatoxin testing is particularly difficult because it has been established that the incidence of significantly contaminated nuts is usually very low, in the order of 1 nut in 10,000 to 1 nut in 30,000 (or more). This means that even a 30 kg sample, as recommended by the European Union, may only contain a single contaminated nut. However, pistachio nuts can contain very high levels of aflatoxin, up to 1,000,000 ng, so a single contaminated nut could give a level of 33 µg/kg (ppb) in a 30 kg sample.

The nuts are exported in a number of forms including: whole raw nuts for further processing; roasted and salted nuts with or without red staining; and kernels for the food manufacturing industry.

This example is based on a blend of two studies that covered the pistachio commodity system across the whole Region. The wet process of de-hulling followed by segregation by flotation is illustrated here. Although this 're-wetting' introduces a potential risk of further aflatoxin contamination, this can be eliminated by the correct use of efficient mechanical driers. If such driers are NOT available, then a dry process should be used.

Pre-requisite programmes that must be in place include: GAP, GSP, GMP, and the more specific 'Codex Recommended International Code of Hygienic Practice for Tree Nuts (CAC/ RCP 6-1972) (FAO/WHO,1994b)'. The latter covers basic hygienic requirements for orchard, farm processing, and commercial processing.

Task 1 - The HACCP team

An appropriate HACCP team will include: HACCP specialist, factory manager, factory quality assurance manager, mycotoxicologist, mycologist, edible nut specialist, procurement agency manager, laboratory manager, socio-economist, and representatives of the Department of Agriculture and of the private sector farming, domestic trading and export sectors.

Tasks 2 and 3 - Product description and intended use.

The product description and intended use is given in Table 13.

Tasks 4 and 5: The Commodity Flow Diagram (CFD), Verified

The CFD was established and verified, as in Figure 13.

Task 6: Mycotoxin hazard analysis and identification of possible control measures.

Hazard Analysis

a) Identification of mycotoxin hazard

Aflatoxin is the only mycotoxin hazard for which the EU and US have regulatory limits for edible nuts; consequently, it is the only mycotoxin considered.

b) Identification of steps in the Commodity Flow Diagram (CFD) where mycotoxin contamination is most likely to occur.

Step 1: On farm, pre-harvest.

This is the step when most aflatoxin contamination will usually occur , and is associated with damage to the hull (Doster, M. A., 1995). This damage is either caused by early-splitting when the hull cannot accommodate the splitting of the shell within, or by growing-split. Subsequent invasion of split-nuts by insects, particularly the navel orangeworm, compounds the problem.

Step 2: On-farm harvesting.

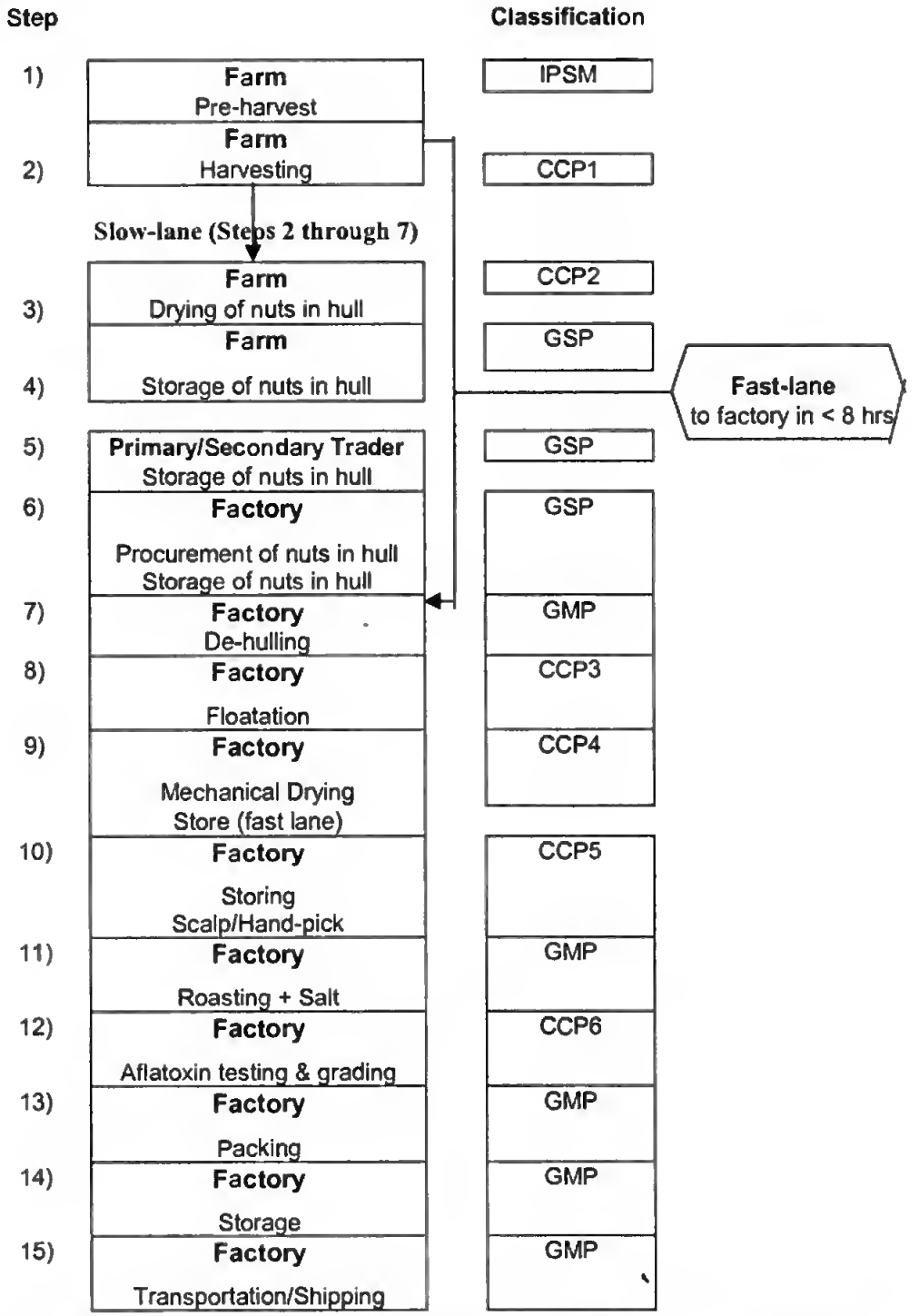
Aflatoxin contamination may occur at this step if pistachio nuts are allowed to fall naturally and remain on the floor, ungathered, for an extended period.
Pistachio nuts can be pre-disposed to subsequent mould and aflatoxin contamination when harvesting is achieved by shaking the tree. This can cause tearing of the hull which can let in mould spores.

Table 13. Product description and intended use of pistachio nuts

Name of Product	Confectionery Pistachio Nuts
Description	Pistachio nuts, partially split, roasted and salted
Customer specification	Ready-split, white or red-stained shells. No obviously mouldy nuts and no rancid nuts. Aflatoxin limit, e.g. 2 µg/kg B_1, 4 µg/kg total for EU.
Conditions of storage (finished product)	Ambient temperature, but < or =10°C preferred for long-term storage.
Shelf Life	1 year
Intended use	Confectionery snack food
Packaging	Plastic-foil laminate, vacuum sealed or over nitrogen
Target Consumer	European and US

Target level is < or = 2 µg/kg aflatoxin B_1 for export to the EU and < or = 20 µg/kg total aflatoxin for export to the US.

Fig. 13. HACCP Process Flow-diagram: Pistaccio Nuts, roasted - West Asia



Note: A factory will usually either be procuring and processing nuts from the "slow-lane" or the "fast-lane", not both at the same time.

Steps 3 to 8 are the 'slow-lane' process flow:

Step 3: Farm drying of nuts in hull.

This is a step designed to reduce aflatoxin contamination by drying to a 'safe' moisture level before storage.

Step 4: Farm, storage of nuts in hull

Aflatoxin contamination is possible if nuts are put into store at an 'unsafe' moisture content, particularly if nuts with damaged hulls are stored.

Step 5: Primary and Secondary Trader

Aflatoxin contamination is possible, particularly if purchased directly from a farmer at harvest.

Step 6: Factory Procurement and storage of nuts in hull (slow lane)

The risk of aflatoxin contamination is low at this step because the nuts have usually dried to a 'safe' moisture content by this time.

Step 7: Factory de-hulling

1. Slow lane

De-hulled nuts are usually further processed without delay, and there is no risk of aflatoxin contamination at this step.

2. Fast lane (Nuts direct from farms)

The wet-process of de-hulling can pre-dispose nuts to subsequent aflatoxin contamination (see step 10).

Step 8: Factory floatation

Levels of aflatoxin will be significantly reduced at this step.

Step 9: Factory drying (and storage in the fast lane)

No aflatoxin contamination is likely at this step, provided that drying to a safe moisture content can be completed within 24 hours. Inadequate drying will leave nuts in the fast lane process susceptible to aflatoxin contamination during subsequent storage.

Step 10: Factory sorting

Levels of aflatoxin will be significantly reduced at this step.

Step 11: Roasting and salting

No aflatoxin contamination is possible at this step. Roasting would be expected to reduce levels of aflatoxin.

Step 12: Factory, aflatoxin testing and grading

No risk of aflatoxin contamination at this step

Step 13: Factory packing

No risk of aflatoxin contamination, but inappropriate packing may make the nuts susceptible to future contamination if re-wetting occurs.

Step 14: Factory storage of finished product

Such storage is usually only short-term and there is negligible risk of aflatoxin contamination.

Step 15: Factory export

No aflatoxin contamination is likely at this stage, or during subsequent transportation. It is very important to select packages, for each consignment, which meet the customer's aflatoxin specification.

c) Possible Mycotoxin Control Measures

The most effective preventative control is to dry pistachio nuts to a water activity of 0.82 for short-term or 0.70 for long-term storage to prevent mould growth and aflatoxin contamination. At

25°C, these critical water activities translate to moisture contents of approximately 10% and 5 to 7 % respectively (Olsen, M., 1999).

Removal of aflatoxin contaminated nuts by means of physical segregation is the most effective control measure for reducing levels of aflatoxin in a batch to an acceptable level. Examples of segregation techniques are: hand-pick sorting, floatation, sorting by size, and the rejection of excessively contaminated batches.

Tasks 7 to 10: Development of a HACCP Plan

A worksheet summarising the HACCP Plan for pistachio nuts is given in Table 14, and development of the plan at each step is discussed below.

Step 1: Farm, preharvest – IPSM/GAP

Pre-harvest aflatoxin contamination can be reduced by applying Integrated Phytosanitary Management (IPSM) (Boutrif, E., 1998) which seeks to minimise the mould spore count in the orchard and minimise the chances of insect attack. Removal or burial of tree litter has been suggested as a measure that would significantly reduce spore count.

Step 2: on-farm harvesting – CCP1

This step is classified as a CCP, with segregation and removal of nuts with damaged hulls as the control measure. This CCP will reduce the mould hazard to acceptable levels, and remove a very high proportion of aflatoxin that has been produced pre-harvest.

The critical limit will be set at <=1% damaged nuts remaining after inspection and the CCP will be monitored by visual observation.

Post-harvest aflatoxin contamination can occur as a result of harvesting by shaking the tree. This can cause tearing of the hull which can let in spores and allow aflatoxin to be produced. Nuts which are allowed to fall to the ground naturally may also become mouldy if they are left on the ground for an extended period. It is considered GAP to place a plastic sheet or tarpaulin under a tree due for harvesting. Pistachios are then either harvested by hand or natural fallers are collected daily.

Steps 3 to 8 are the 'slow-lane' process flow.

Step 3: Farm, drying of nuts in hull – CCP2

This step is identified as a CCP with drying to a safe moisture content as the control measure. Research is required to determine the moisture content of a nut in hull that corresponds with a water activity of 0.7 at 25°C. The critical limits will then be set in terms of number of days sun-drying required to achieve the safe moisture content.

Step 4: Farm, storage of nuts in hull - GSP

Sound nuts at a safe moisture content will store well provided that GSP is in place.

Step 5: Primary and Secondary Trader - GSP

Good storage practice is necessary to prevent re-wetting of the pistachios and to control insect damage.

Step 6: Factory, procurement and storage of nuts in hull (slow lane) – GMP/ GSP

It is considered GMP to procure high-quality nuts, with a low percentage of damaged hulls. A premium price for such nuts will encourage traders and farmers to produce this quality, and rejection or a low price will discourage production of poor quality nuts.

Good storage practice will enable long-term storage of nuts in hull, if required. Regular fumigation will be required to control insects.

Step 7: Factory de-hulling - GMP

The wet process should only be used if the factory has a reliable, efficient mechanical drier for use at Step 10. Failing this, the dry process should be used.

Step 8: Factory floatation - CCP3

This step has been identified as a CCP with the removal of nuts that float as the control measure. Studies indicate (Schatzki, T., 1996) that in the order of 40% of aflatoxin will be removed. This CCP, in conjunction with subsequent CCPs will reduce levels of aflatoxin to an acceptable level

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in a high proportion of batches. Monitoring will be by visual inspection, using trained staff, to check that <1% 0f floating material remains.

Step 9: Factory drying – CCP4

This is a CCP for the fast-lane process, when de-hulled nuts are stored (or exported) prior to further processing. Slow-lane nuts are moved on to step 11 without delay and this is not a CCP for this process.

The control measure is to dry the nuts to a moisture content of 10% within 24 hours, for short-term storage, and to 6% within 48 hours for long-term storage. Critical limits will be set for the operating temperature of the drier, and the dwell time in the drier. The critical limits for temperature will be monitored by regular, or continuous, temperature reading obtained using a calibrated thermometer.

Step 10: Factory sorting – CCP5

The control measure at this CCP is to remove small nuts (scalp) and hand-pick sort (HPS) to remove damaged nuts. Studies in the USA (Schatzki, T. F., 1996) indicate that small nuts (>30 nuts per ounce) contain between 20 to 40% of aflatoxin originally present in a batch. After removal of small nuts, subsequent hand-pick sorting to remove damaged nuts (particularly insect damaged) and nuts having pieces of hull still adhering to the shell, will further substantially reduce levels of aflatoxin. When applied to fast-lane nuts, the hand-pick sorting is extended to include the removal of nuts with stained shells, and this will make the control measure even more effective in reducing levels of aflatoxin.

Monitoring of this CCP is achieved by visual observation by staff trained to detect an unacceptable level (e.g. 5%) of damaged or discoloured nuts remaining after HPS sorting.

Step 11: Roasting and salting - GMP

No aflatoxin contamination is possible at this step. Roasting will reduce levels of aflatoxin, perhaps by of the order of 20%.

Step 12: Factory, aflatoxin testing and grading – CCP6

This step will be a CCP initially, but as aflatoxin control improves the HACCP team may well use aflatoxin testing at this step for verification purposes only.

The control measure is to carry out aflatoxin testing on every batch and to grade the batches accordingly. Unfortunately, a large sample size of 30 kg is required, as explained in the Introduction. The critical limits will be set at the customer specification, e.g. 2 µg/kg aflatoxin B_1 for the EU and 20 µg/kg aflatoxin B_1 for the US. The critical limit is monitored by performing rapid, semi-quantitative aflatoxin testing on representative samples. Alternatively, samples could be sent to an accredited laboratory for certification purposes.

Step 13: Factory packing - GMP

Appropriate packaging is required to prevent re-wetting and to retain other quality factors. Air-tight packaging, either under vacuum or over nitrogen is preferred.

Step 14: Factory storage of finished product -GMP

Storage at ambient temperatures is adequate for short-term storage, but longer term storage requires a reduced temperature of less than or equal to 10°C.

Step 15: Factory export - GMP

Batches of pistachios are selected for export to meet the customers' aflatoxin specification, using information gained at step 12.

Task 11: Establish verification procedures

The HACCP Plan will be audited quarterly, and amended as necessary.

Task 12: Establish documentation and record keeping

The HACCP Plan will be fully documented, and appropriate records will be kept at each CCP.

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Table 14. HACCP Plan Worksheet, Pistachio nuts, roasted, produced in West Asia.

Process Step	Description of hazard	Possible Control Measures	Control	Critical Limits	Monitoring Procedures	Corrective Actions	Records
1 Farm Pre-harvest	Mould	Select resistant variety (long-term); Reduce spore count in air and soil	IPSM	Remove >95% tree litter	Visual observation	e.g. remove or bury litter	Farmer
2 Farm Harvesting	Mould	Remove early splits and/ or insect damaged nuts by farmer inspection use tarpaulin on ground transport directly to factory within 8 hours of harvest (fast lane only)	CCP1 GAP GAP	<1% damaged nuts remaining	Visual observation (premium for <1% early splits)	Re-sort batch	Farmer
3 Farm Drying (Slow-lane)	Mould	Dry thoroughly before storage of hulls ('safe' moisture content of hulls to be determined by research)	CCP2	Research needed: e.g. 3 days sun-drying?	Timing of drying period	Extend drying period Remove mouldy nuts	Farmer
4 Farm Storage of nuts in hull	Mould Insects	Raise from the ground & have a sound roof Insecticide treatment	GSP GSP				
5 Primary/secondary storage of nuts in hull	Mould Insects	Raise from the ground & have a sound roof insecticide treatment	GSP GSP				
6 Factory Procurement/Storage	Mould Mould Insects	Procure nuts with sound hulls (offer premium for batches containing <1% splits) Raise from the ground & have a sound roof Insecticide	GMP GSP GSP				
7							

Factory Dehulling	Aflatoxin	Do not re-use water	GMP				
8 Factory Floatation	Aflatoxin	Remove floating material which reduces aflatoxin levels by ~70%	CCP3	Remove >99% floating material	Visual observation	repeat removal process	Factory records
9 Factory Drying	Aflatoxin	Dry nuts uniformly to 12% m.c.	CCP4	Temperature and time parameters e.g.82°C +/- 2°C for 3 hours +/- 3 minutes	Chart recording Timer	Repair fault/ re-dry nuts or discard nuts if delay	Factory records
10 Factory Sorting	Aflatoxin	Scalp, remove very small nuts, >30 nuts per ounce or 106 nuts per 100 g Remove discoloured, or shrivelled or damaged nuts	CCP5	Remove >99% of small nuts Remove >95% of undesirable nuts	Grading check Grading check	Repeat scalp Repeat sorting	Factory records Factory records
11 Factory Roasting and salting			GMP				
12 Factory Aflatoxin testing & grading	Aflatoxin	Determine aflatoxin level in batch by collecting representative 30 kg sample from the conveyor belt and analyse for aflatoxin	CCP6	< or = 2 µg/kg B1 for EU < or = 20 µg/kg total aflatoxin for US	Aflatoxin testing using rapid kits	Reject batches not meeting aflatoxin requirement	Factory records
13 Factory Packing	Aflatoxin	Air-tight packaging, preferably vacuum packed, or over nitrogen	GMP				
14 Factory Storage	Aflatoxin	Ambient temperature, but < or =10°C for long-term storage	GSP				
15 Factory Export	Aflatoxin	Select packets that meet the customer's aflatoxin specification using data from Step 12	GMP				

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52 Add. 2 Compendium of food additive specifications – Addendum 2, 1993 (E)
52 Add. 3 Compendium of food additive specifications – Addendum 3, 1995 (E)
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52 Add. 5 Compendium of food additive specifications – Addendum 5, 1997 (E)
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The manual provides guidance on the application of the Hazard Analysis Critical Control Point (HACCP) approach to the prevention and control of mycotoxin contamination of foods and feed. After a brief introduction on the nature of mycotoxins and their effects on human and animal health, the document describes the HACCP system, as defined by the Codex Alimentarius Commission. Six examples (yellow maize kernels, maize-based animal feed, copra cake and meal, commercially produced peanut butter, apple juice and pistachio nuts) illustrate how the HACCP approach can be applied to prevent and control mycotoxin contamination.

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